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 * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *
FILE 'HOME' ENTERED AT 17:48:18 ON 19 NOV 2003
=> file biosis,caba,caplus,embase,japio,lifesci,medline,scisearch,uspatfull
=> e reed steven g/au
E1        1   REED STEVEN C SR/AU
E2        2   REED STEVEN F/AU
E3      403 --> REED STEVEN G/AU
E4      246   REED STEVEN I/AU
E5        1   REED STEVEN IRA/AU
E6        4   REED STEVEN J/AU
E7        1   REED STEVEN K/AU
E8        3   REED STEVEN L/AU
E9        1   REED STEVEN M/AU
E10       7   REED STEVEN P/AU
E11       1   REED STEVEN S/AU
E12       1   REED STEVEN SCOTT/AU

=> s e3 and tuberculosis
L1      124 "REED STEVEN G"/AU AND TUBERCULOSIS

=> dup rem l1
PROCESSING COMPLETED FOR L1
L2      102 DUP REM L1 (22 DUPLICATES REMOVED)

=> s l2 and tbh9fl
L3        0 L2 AND TBH9FL

=> s l2 and (TbH-9-fl)
L4        2 L2 AND (TBH-9-FL)

=> d bib ab kwic 1-
YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y

L4  ANSWER 1 OF 2  USPATFULL on STN
AN    2003:206886 USPATFULL
TI    Compounds and methods for immunotherapy and diagnosis of
      ***tuberculosis***
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PI    US 2003143243     A1   20030731
AI    US 2002-84843     A1   20020225 (10)
RLI   Continuation of Ser. No. US 1998-72967, filed on 5 May 1998, PENDING
      Continuation-in-part of Ser. No. US 1998-25197, filed on 18 Feb 1998,
      ABANDONED Continuation-in-part of Ser. No. US 1997-942578, filed on 1
      Oct 1997, ABANDONED Continuation-in-part of Ser. No. US 1997-818112,
      filed on 13 Mar 1997, GRANTED, Pat. No. US 6290969 Continuation-in-part
      of Ser. No. US 1996-730510, filed on 11 Oct 1996, ABANDONED
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Continuation-in-part of Ser. No. US 1996-680574, filed on 12 Jul 1996, ABANDONED Continuation-in-part of Ser. No. US 1996-659683, filed on 5 Jun 1996, ABANDONED Continuation-in-part of Ser. No. US 1996-620874, filed on 22 Mar 1996, ABANDONED Continuation-in-part of Ser. No. US 1995-533634, filed on 22 Sep 1995, ABANDONED Continuation-in-part of Ser. No. US 1995-523436, filed on 1 Sep 1995, ABANDONED

PRAI WO 1996-US14674 19960830

DT Utility

FS APPLICATION

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CLMN Number of Claims: 37

ECL Exemplary Claim: 1

DRWN 19 Drawing Page(s)

LN.CNT 9257

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compounds and methods for inducing protective immunity against ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one immunogenic portion of one or more M. ***tuberculosis*** proteins and DNA molecules encoding such polypeptides. Such compounds may be formulated into vaccines and/or pharmaceutical compositions for immunization against M. ***tuberculosis*** infection, or may be used for the diagnosis of ***tuberculosis*** .

TI Compounds and methods for immunotherapy and diagnosis of ***tuberculosis***

IN ***Reed, Steven G.*** , Bellevue, WA, UNITED STATES

AB Compounds and methods for inducing protective immunity against ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one immunogenic portion of one or more M. ***tuberculosis*** proteins and DNA molecules encoding such polypeptides. Such compounds may be formulated into vaccines and/or pharmaceutical compositions for immunization against M. ***tuberculosis*** infection, or may be used for the diagnosis of ***tuberculosis*** .

SUMM [0002] The present invention relates generally to detecting, treating and preventing Mycobacterium ***tuberculosis*** infection. The invention is more particularly related to polypeptides comprising a Mycobacterium ***tuberculosis*** antigen, or a portion or other variant thereof, and the use of such polypeptides for diagnosing and vaccinating against Mycobacterium ***tuberculosis*** infection.

SUMM [0003] ***Tuberculosis*** is a chronic, infectious disease, that is generally caused by infection with Mycobacterium ***tuberculosis*** . It is a major disease in developing countries, as well as an increasing problem in developed areas of the world,. . .

SUMM [0004] Although ***tuberculosis*** can generally be controlled using extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease.. . .

SUMM [0005] Inhibiting the spread of ***tuberculosis*** requires effective vaccination and accurate, early diagnosis of the disease. Currently, vaccination with live bacteria is the most efficient method. . .

SUMM [0006] While macrophages have been shown to act as the principal effectors of M ***tuberculosis*** immunity, T cells are the predominant inducers of such immunity. The essential role of T cells in

protection against M. ***tuberculosis*** infection is illustrated by the frequent occurrence of M. ***tuberculosis*** in AIDS patients, due to the depletion of CD4 T cells associated with human immunodeficiency virus (HIV) infection. Mycobacterium-reactive CD4. . . that 1,25-dihydroxy-vitamin D3, either alone or in combination with IFN-.gamma. or tumor necrosis factor-alpha, activates human macrophages to inhibit M. ***tuberculosis*** infection. Furthermore, it is known that IFN-.gamma. stimulates human macrophages to make 1,25-dihydroxy-vitamin D3. Similarly, IL-12 has been shown to play a role in stimulating resistance to M. ***tuberculosis*** infection. For a review of the immunology of M. ***tuberculosis*** infection see Chan and Kaufmann in ***Tuberculosis*** : Pathogenesis, Protection and Control, Bloom (ed.), ASM Press, Washington, D.C., 1994.

SUMM [0007] Accordingly, there is a need in the art for improved vaccines and methods for preventing, treating and detecting ***tuberculosis*** . The present invention fulfills these needs and further provides other related advantages.

SUMM [0008] Briefly stated, this invention provides compounds and methods for preventing and diagnosing ***tuberculosis*** . In one aspect, polypeptides are provided comprising an immunogenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. In one embodiment of. . .

SUMM [0022] In a related aspect, polypeptides are provided comprising an immunogenic portion of an M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, the antigen having one. . .

SUMM [0026] In another embodiment, the soluble M. ***tuberculosis*** antigen comprises an amino acid sequence encoded by a DNA sequence selected from the group consisting of the sequences recited. . .

SUMM [0027] In a related aspect, the polypeptides comprise an immunogenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, wherein the antigen comprises. . .

SUMM . . . provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, an inventive polypeptide and a known M. ***tuberculosis*** antigen.

SUMM [0032] In further aspects of this invention, methods and diagnostic kits are provided for detecting ***tuberculosis*** in a patient. The methods comprise contacting dermal cells of a patient with one or more of the above polypeptides. . .

SUMM [0033] In yet other aspects, methods are provided for detecting ***tuberculosis*** in a patient, such methods comprising contacting dermal cells of a patient with one or more polypeptides encoded by a. . .

DRWD . . . B illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a first and a second M. ***tuberculosis*** -immune donor, respectively, by the 14 Kd, 20 Kd and 26 Kd antigens described in Example 1.

DRWD [0036] FIG. 2 illustrates the stimulation of proliferation and interferon-.gamma. production in T cells derived from an M. ***tuberculosis*** -immune individual by the two representative polypeptides TbRa3 and TbRa9.

DRWD [0037] FIGS. 3A-D illustrate the reactivity of antisera raised against secretory M. ***tuberculosis*** proteins, the known M.

tuberculosis antigen 85b and the inventive antigens Tb38-1 and TbH-9, respectively, with M. ***tuberculosis*** lysate (lane 2), M. ***tuberculosis*** secretory proteins (lane 3), recombinant Tb38-1 (lane 4), recombinant TbH-9 (lane 5) and recombinant 85b (lane 5).

DRWD [0038] FIG. 4A illustrates the stimulation of proliferation in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, recombinant TbH-9 and a control antigen, TbRa11.

DRWD [0039] FIG. 4B illustrates the stimulation of interferon-.gamma. production in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, PPD and recombinant TbH-9.

DRWD . . . FIGS. 8A and B illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a first M. ***tuberculosis*** -immune individual by the representative polypeptides XP-1, RDIF6, RDIF8, RDIF10 and RDIF11.

DRWD . . . FIGS. 9A and B illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a second M. ***tuberculosis*** -immune individual by the representative polypeptides XP-1, RDIF6, RDIF8, RDIF10 and RDIF11.

DETD [0190] SEQ ID NO: 154 is the DNA sequence of the M. ***tuberculosis*** antigen 38 kD.

DETD [0191] SEQ ID NO: 155 is the amino acid sequence of the M. ***tuberculosis*** antigen 38 kD.

DETD [0373] SEQ ID NO: 343 is the determined amino acid sequence for a M. ***tuberculosis*** 85b precursor homolog

DETD [0386] As noted above, the present invention is generally directed to compositions and methods for preventing, treating and diagnosing ***tuberculosis*** . The compositions of the subject invention include polypeptides that comprise at least one immunogenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. Polypeptides within the scope of the present invention include, but are not limited to, immunogenic soluble M. ***tuberculosis*** antigens. A "soluble M. ***tuberculosis*** antigen" is a protein of M. ***tuberculosis*** origin that is present in M. ***tuberculosis*** culture filtrate. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e.,. . . . entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native M. ***tuberculosis*** antigen or may be heterologous, and such sequences may (but need not) be immunogenic.

DETD . . . from the group of T cells, NK cells, B cells and macrophages, where the cells are derived from an M. ***tuberculosis*** -immune individual. Polypeptides comprising at least an immunogenic portion of one or more M. ***tuberculosis*** antigens may generally be used to detect ***tuberculosis*** or to induce protective immunity against ***tuberculosis*** in a patient.

DETD . . . be identified by evaluating a modified polypeptide for the ability to generate antibodies that detect the presence or absence of ***tuberculosis*** . Such modified sequences may be prepared and tested using, for example, the representative procedures described herein.

DETD . . . polypeptide" is a polypeptide comprising at least one of the above immunogenic portions and one or more additional immunogenic M. ***tuberculosis*** sequences, which are joined via a peptide linkage into a single amino acid chain. The sequences may be joined directly. . .

DETD [0393] In general, M. ***tuberculosis*** antigens, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, soluble antigens may be isolated from M. ***tuberculosis*** culture filtrate by procedures known to those of ordinary skill in the art, including anion-exchange and reverse phase chromatography. Purified. . .

DETD . . . vector and expressed in an appropriate host. DNA molecules encoding soluble antigens may be isolated by screening an appropriate M. ***tuberculosis*** expression library with anti-sera (e.g., rabbit) raised specifically against soluble M. ***tuberculosis*** antigens. DNA sequences encoding antigens that may or may not be soluble may be identified by screening an appropriate M. ***tuberculosis*** genomic or cDNA expression library with sera obtained from patients infected with M. ***tuberculosis*** . Such screens may generally be performed using techniques well known to those of ordinary skill in the art, such as. . .

DETD [0395] DNA sequences encoding soluble antigens may also be obtained by screening an appropriate M. ***tuberculosis*** cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of. . .

DETD [0396] Alternatively, genomic or cDNA libraries derived from M. ***tuberculosis*** may be screened directly using peripheral blood mononuclear cells (PBMCs) or T cell lines or clones derived from one or more M. ***tuberculosis*** -immune individuals. In general, PBMCs and/or T cells for use in such screens may be prepared as described below. Direct library. . . of expressed recombinant proteins for the ability to induce proliferation and/or interferon-.gamma. production in T cells derived from an M. ***tuberculosis*** -immune individual. Alternatively, potential T cell antigens may be first selected based on antibody reactivity, as described above.

DETD . . . cytokine production (ie., interferon-.gamma. and/or interleukin-12 production) in T cells, NK cells, B cells and/or macrophages derived from an M. ***tuberculosis*** -immune individual. The selection of cell type for use in evaluating an immunogenic response to a antigen will, of course, depend. . . the desired response. For example, interleukin-12 production is most readily evaluated using preparations containing B cells and/or macrophages. An M. ***tuberculosis*** -immune individual is one who is considered to be resistant to the development of ***tuberculosis*** by virtue of having mounted an effective T cell response to M. ***tuberculosis*** (i.e., substantially free of disease symptoms). Such individuals may be identified based on a strongly positive (i.e., greater than about 10 mm diameter induration) intradermal skin test response to ***tuberculosis*** proteins (PPD) and an absence of any signs or symptoms of ***tuberculosis*** disease. T cells, NK cells, B cells and macrophages derived from M. ***tuberculosis*** -immune individuals may be prepared using methods known to those of ordinary skill in the art. For example, a preparation of. . . individual mycobacterial proteins, may be employed. Such T cell clones may be generated by, for example, culturing PBMCs from M. ***tuberculosis*** -immune individuals with mycobacterial proteins for a period of 2-4 weeks. This allows expansion of only the mycobacterial protein-specific T cells,. . . (i.e., interferon-.gamma. and/or interleukin- 12 production) performed using T cells, NK cells, B cells and/or macrophages derived from an M. ***tuberculosis*** -immune individual

are considered immunogenic. Such assays may be performed, for example, using the representative procedures described below. Immunogenic portions of. . .

DETD . . . and/or interleukin-12 production) in T cells, NK cells, B cells and/or macrophages derived from at least about 25% of M. ***tuberculosis*** -immune individuals. Among these immunogenic antigens, polypeptides having superior therapeutic properties may be distinguished based on the magnitude of the responses. . . proliferation and/or cytokine production in vitro in cells derived from more than about 25% of individuals that are not M. ***tuberculosis*** -immune, thereby eliminating responses that are not specifically due to M. ***tuberculosis*** -responsive cells. Those antigens that induce a response in a high percentage of T cell, NK cell, B cell and/or macrophage preparations from M. ***tuberculosis*** -immune individuals (with a low incidence of responses in cell preparations from other individuals) have superior therapeutic properties.

DETD [0401] Antigens with superior therapeutic properties may also be identified based on their ability to diminish the severity of M. ***tuberculosis*** infection in experimental animals, when administered as a vaccine. Suitable vaccine preparations for use on experimental animals are described in. . .

DETD . . . identified based on the ability to elicit a response in an intradermal skin test performed on an individual with active ***tuberculosis*** , but not in a test performed on an individual who is not infected with M. ***tuberculosis*** . Skin tests may generally be performed as described below, with a response of at least 5 mm induration considered positive.

DETD [0404] Portions and other variants of M. ***tuberculosis*** antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally. . .

DETD [0408] In certain specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a soluble M. ***tuberculosis*** antigen having one of the following N-terminal sequences, or a variant thereof that differs only in conservative substitutions and/or modifications:

DETD [0422] In a further specific embodiment, the subject invention discloses polypeptides comprising at least an immunogenic portion of an M. ***tuberculosis*** antigen having one of the following N-terminal sequences, or a variant thereof that differs only in conservative substitutions and/or modifications:

DETD [0426] In other specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a soluble M. ***tuberculosis*** antigen (or a variant of such an antigen) that comprises one or more of the amino acid sequences encoded by. . .

DETD [0427] In further specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a M. ***tuberculosis*** antigen (or a variant of such an antigen), which may or may not be soluble, that comprises one or more. . .

DETD [0428] In the specific embodiments discussed above, the M. ***tuberculosis*** antigens include variants that are encoded by DNA sequences which are substantially homologous to one or more of DNA sequences. . .

DETD . . . comprising a first and a second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known M.

tuberculosis antigen, such as the 38 kD antigen described in Andersen and Hansen, Infect. Immun. 57:2481-2488, 1989, (Genbank Accession No. M30046). . .

DETD . . . or more of the above polypeptides or fusion proteins (or DNA molecules encoding such polypeptides) to induce protective immunity against ***tuberculosis*** in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be. . . may be free of detectable disease and/or infection. In other words, protective immunity may be induced to prevent or treat ***tuberculosis*** .

DETD . . . adjuvant or a liposome (into which the polypeptide is incorporated). Such pharmaceutical compositions and vaccines may also contain other M. ***tuberculosis*** antigens, either incorporated into a combination polypeptide or present within a separate polypeptide.

DETD . . . above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known M. ***tuberculosis*** antigen, such as the 38 kD antigen described above. For example, administration of DNA encoding a polypeptide of the present. . .

DETD . . . described above, is capable of raising an immune response in an immunized patient sufficient to protect the patient from M. ***tuberculosis*** infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced in situ. . .

DETD . . . aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune responses, such as lipid A, Bortadella pertussis or Mycobacterium ***tuberculosis*** . Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Freund's Complete Adjuvant (Difco Laboratories) and Merck Adjuvant. . .

DETD [0440] In another aspect, this invention provides methods for using one or more of the polypeptides described above to diagnose ***tuberculosis*** using a skin test. As used herein, a "skin test" is any assay performed directly on a patient in which. . .

DETD . . . than about 0.5 cm in diameter, preferably greater than about 1.0 cm in diameter, is a positive response, indicative of ***tuberculosis*** infection, which may or may not be manifested as an active disease.

DETD Purification and Characterization of Polypeptides From M. ***tuberculosis*** Culture Filtrate

DETD [0445] This example illustrates the preparation of M. ***tuberculosis*** soluble polypeptides from culture filtrate. Unless otherwise noted, all percentages in the following example are weight per volume.

DETD [0446] M. ***tuberculosis*** (either H37Ra, ATCC No. 25177, or H37Rv, ATCC No. 25618) was cultured in sterile GAS media at 37.degree. C. for. . .

DETD [0467] Additional soluble antigens were isolated from M. ***tuberculosis*** culture filtrate as follows. M. ***tuberculosis*** culture filtrate was prepared as described above. Following dialysis against Bis-Tris propane buffer, at pH 5.5, fractionation was performed using. . .

DETD . . . sequences that encode the antigens designated as (a), (c), (d) and (g) above were obtained by screening a genomic M. ***tuberculosis*** library using .sup.32P end labeled degenerate oligonucleotides corresponding to the N-terminal sequence and containing

M. ***tuberculosis*** codon bias. The screen performed using a probe corresponding to antigen (a) above identified a clone having the sequence provided. . .

DETD . . . was amplified from genomic DNA using the sequence obtained from GENBANK. This sequence was then used to screen the M. ***tuberculosis*** library described below in Example 2 and a full length copy of the M. ***tuberculosis*** homologue was obtained (SEQ ID No. 99).

DETD [0478] The amino acid sequence for antigen (j) was found to be homologous to a known M. ***tuberculosis*** protein translated from a DNA sequence. To the best of the inventors' knowledge, this protein has not been previously shown. . .

DETD Use of Patient Sera to Isolate M. ***tuberculosis*** Antigens

DETD [0481] This example illustrates the isolation of antigens from M. ***tuberculosis*** lysate by screening with serum from M. ***tuberculosis*** -infected individuals.

DETD [0482] Dessicated M. ***tuberculosis*** H37Ra (Difco Laboratories) was added to a 2% NP40 solution, and alternately homogenized and sonicated three times. The resulting suspension. . . Centriprep 10 (Amicon, Beverley, Mass.) and then screened by Western blot for serological activity using a serum pool from M. ***tuberculosis*** -infected patients which was not immunoreactive with other antigens of the present invention.

DETD [0486] A DNA sequence that encodes the antigen designated as (m) above was obtained by screening a genomic M. ***tuberculosis*** Erdman strain library using labeled degenerate oligonucleotides corresponding to the N-terminal sequence of SEQ ID NO: 137. A clone was. . . NO: 204. Comparison of these sequences with those in the genebank revealed some similarity to sequences previously identified in M. ***tuberculosis*** and M. bovis.

DETD Preparation of DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD [0487] This example illustrates the preparation of DNA sequences encoding M. ***tuberculosis*** antigens by screening a M. ***tuberculosis*** expression library with sera obtained from patients infected with M. ***tuberculosis*** , or with anti-sera raised against soluble M. ***tuberculosis*** antigens.

DETD [0488] A. Preparation of M. ***tuberculosis*** Soluble Antigens Using Rabbit Anti-Sera Raised Against M. ***tuberculosis*** Supernatant

DETD [0489] Genomic DNA was isolated from the M. ***tuberculosis*** strain H37Ra. The DNA was randomly sheared and used to construct an expression library using the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Rabbit anti-sera was generated against secretory proteins of the M. ***tuberculosis*** strains H37Ra, H37Rv and Erdman by immunizing a rabbit with concentrated supernatant of the M. ***tuberculosis*** cultures. Specifically, the rabbit was first immunized subcutaneously with 200 .mu.g of protein antigen in a total volume of 2. . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD [0490] Thirty two clones were purified. Of these, 25 represent sequences that have not been previously identified in human M. ***tuberculosis*** . Recombinant antigens were expressed and purified antigens used in the immunological analysis described in Example 1. Proteins were induced by. . .

DETD . . . ID Nos. 76, 68, 70, 75) show some homology to sequences previously identified in Mycobacterium leprae but not in M. ***tuberculosis*** . TbRA2A was found to be a lipoprotein, with a six residue lipidation sequence being located adjacent to a hydrophobic secretory sequence. TbRA11, TbRA26, TbRA28 and TbDPEP (SEQ ID Nos.: 65, 73, 74, 53) have been previously identified in M. ***tuberculosis*** . No significant homologies were found to TbRA1, TbRA3, TbRA4, TbRA9, TbRA10, TbRA13, TbRA17, TbRa19, TbRA29, TbRA32, TbRA36 and the overlapping. . .

DETD . . . results of PBMC proliferation and interferon-.gamma. assays performed on representative recombinant antigens, and using T-cell preparations from several different M. ***tuberculosis*** -immune patients, are presented in Tables 2 and 3, respectively.

TABLE 2

RESULTS OF PBMC PROLIFERATION TO
REPRESENTATIVE SOLUBLE ANTIGENS

Patient

Antigen 1 2. . .

DETD [0495] These results indicate that these soluble antigens can induce proliferation and/or interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** -immune individual.

DETD [0496] B. Use of Sera From Patients Having Pulmonary or Pleural ***Tuberculosis*** to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD . . . DNA library described above, and an additional H37Rv library, were screened using pools of sera obtained from patients with active ***tuberculosis*** . To prepare the H37Rv library, M. ***tuberculosis*** strain H37Rv genomic DNA was isolated, subjected to partial Sau3A digestion and used to construct an expression library using the. . . and TbH=high reactivity) in both ELISA and immunoblot format. A fourth pool of sera from seven patients with active pulmonary ***tuberculosis*** was also employed. All of the sera lacked increased reactivity with the recombinant 38 kD M. ***tuberculosis*** H37Ra phosphate-binding protein.

DETD . . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD [0499] Thirty two clones were purified. Of these, 31 represented sequences that had not been previously identified in human M. ***tuberculosis*** . Representative sequences of the DNA molecules identified are provided in SEQ ID Nos.: 26-51 and 105. Of these, TbH-8-2 (SEQ.. . . of the open reading frame for the antigen ESAT-6 previously identified in M. bovis (Acc. No. U34848) and in M. ***tuberculosis*** (Sorensen et al., Infec. Immun. 63:1710-1717, 1995).

DETD . . . sequence of Tb38-1F3 is presented in SEQ. ID. NO. 117. A TbH-9 probe identified three clones in the H37Rv library: ***TbH*** - ***9*** - ***FL*** (SEQ. ID NO. 106), which may be the homologue of TbH-9 (R37Ra), TbH-9-1 (SEQ. ID NO. 108), and TbH-9-4 (SEQ.. . .

DETD [0501] Further screening of the M. ***tuberculosis*** genomic DNA library, as described above, resulted in the recovery of ten additional reactive clones, representing seven different genes. One. . . was determined to be identical to the 14Kd alpha crystallin heat shock

protein previously shown to be present in M. ***tuberculosis*** , and a third was determined to be identical to the antigen TbH-8 described above. The determined DNA sequences for the. . . contains the reactive open reading frame), although the 3' end of TbH-29 was found to be identical to the M. ***tuberculosis*** cosmid Y227. TbH-32 and TbH-33 were found to be identical to the previously identified M. ***tuberculosis*** insertion element IS6110 and to the M. ***tuberculosis*** cosmid Y50, respectively. No significant homologies to TbH-30 were found.

DETD . . . and uninduced lysates were run in duplicate on SDS-PAGE and transferred to nitrocellulose filters. Filters were reacted with human M. ***tuberculosis*** sera (1:200 dilution) reactive with TbH and a rabbit sera (1:200 or 1:250 dilution) reactive with the N-terminal 4 Kd. . .

DETD [0503] Positive reaction of the recombinant human M. ***tuberculosis*** antigens with both the human M. ***tuberculosis*** sera and anti-lacZ sera indicate that reactivity of the human M. ***tuberculosis*** sera is directed towards the fusion protein. Antigens reactive with the anti-lacZ sera but not with the human M. ***tuberculosis*** sera may be the result of the human M. ***tuberculosis*** sera recognizing conformational epitopes, or the antigen-antibody binding kinetics may be such that the 2 hour sera exposure in the. . .

DETD [0507] These results indicate that both the inventive M. ***tuberculosis*** antigens and ESAT-6 can induce proliferation and/or interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** -immune individual. To the best of the inventors' knowledge, ESAT-6 has not been previously shown to stimulate human immune responses

DETD . . . help to localize T-cell epitopes within Th38-1 capable of inducing proliferation and interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** immune individual.

TABLE 7

RESULTS OF PBMC PROLIFERATION TO TB38-1 PEPTIDES

Patient

Peptide 1 2 3 4 5 6 7 8 9. . .

DETD [0510] Studies were undertaken to determine whether the antigens TbH-9 and Tb38-1 represent cellular proteins or are secreted into M. ***tuberculosis*** culture media. In the first study, rabbit sera were raised against A) secretory proteins of M. ***tuberculosis*** , B) the known secretory recombinant M. ***tuberculosis*** antigen 85b, C) recombinant Tb38-1 and D) recombinant TbH-9, using protocols substantially the same as that as described in Example 3A. Total M. ***tuberculosis*** lysate, concentrated supernatant of M. ***tuberculosis*** cultures and the recombinant antigens 85b, TbH-9 and Tb38-1 were resolved on denaturing gels, immobilized on nitrocellulose membranes and duplicate. . .

DETD . . . FIGS. 3A-D, respectively, wherein the lane designations are as follows: 1) molecular weight protein standards; 2) 5 .mu.g of M. ***tuberculosis*** lysate; 3) 5 .mu.g secretory proteins; 4) 50 ng recombinant Tb38-1; 5) 50 ng recombinant TbH-9; and 6) 50 ng. . . by an arrow). These results demonstrate that Tb38-1 and TbH-9 are intracellular antigens and are not actively secreted by M.

tuberculosis .

DETD . . . an intracellular antigen was confirmed by determining the reactivity of TbH-9-specific human T cell clones to recombinant TbH-9, secretory M. ***tuberculosis*** proteins and PPD. A TbH-9-specific T cell clone (designated 131TbH-9) was generated from PBMC of a healthy PPD-positive donor. The proliferative response of 131TbH-9 to secretory proteins, recombinant TbH-9 and a control M. ***tuberculosis*** antigen, TbRa11, was determined by measuring uptake of tritiated thymidine, as described in Example 1. As shown in FIG. 4A, the clone 131TbH-9 responds specifically to TbH-9, showing that TbH-9 is not a significant component of M. ***tuberculosis*** secretory proteins. FIG. 4B shows the production of IFN-.gamma. by a second TbH-9-specific T cell clone (designated PPD 800-10) prepared. . . cell clone with secretory proteins, PPD or recombinant TbH-9. These results further confirm that TbH-9 is not secreted by M. ***tuberculosis*** .

DETD [0513] C. Use of Sera From Patients Having Extrapulmonary ***Tuberculosis*** to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD [0514] Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). The resulting library was screened using pools of sera obtained from individuals with extrapulmonary ***tuberculosis*** , as described above in Example 3B, with the secondary antibody being goat anti-human IgG +A +M (H+L) conjugated with alkaline. . .

DETD . . . the exception of the 3' ends of XP2 and XP6 which were found to bear some homology to known M. ***tuberculosis*** cosmids. The DNA sequences for XP27 and XP36 are shown in SEQ ID Nos.: 163 and 164, respectively, with the. . .

DETD . . . described herein, recombinant XP1 was found to stimulate cell proliferation and IFN-.gamma. production in T cells isolated from an M. ***tuberculosis*** -immune donors.

DETD [0518] D. Use of a Lysate Positive Serum Pool From Patients Having ***Tuberculosis*** to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD [0519] Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda Screen expression system (Novagen, Madison, Wis.), as described below in Example 6. Pooled serum obtained from M. ***tuberculosis*** -infected patients and that was shown to react with M. ***tuberculosis*** lysate but not with the previously expressed proteins 38kD, Tb38-1, TbRa3, TbH4, DPEP and TbRa11, was used to screen the. . .

DETD . . . SEQ ID NO: 257-260. The remaining seventeen clones were found to show similarities to unknown sequences previously identified in M. ***tuberculosis*** . The determined 5' cDNA sequences for sixteen of these clones (hereinafter referred to as LSER-1, LSER-3, LSER-4, LSER-5, LSER-6, LSER-8,. . .

DETD [0521] E. Preparation of M. ***tuberculosis*** Soluble Antigens Using Rabbit Anti-Sera Raised Against M. ***tuberculosis*** Fractionated Proteins

DETD [0522] M. ***tuberculosis*** lysate was prepared as described above in Example 2. The resulting material was fractionated by HPLC and the fractions screened by Western blot for serological activity with a serum pool from M. ***tuberculosis*** -infected patients which showed

little or no immunoreactivity with other antigens of the present invention. Rabbit anti-sera was generated against the most reactive fraction using the method described in Example 3A . The anti-sera was used to screen an M. ***tuberculosis*** Erdman strain genomic DNA expression library prepared as described above. Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones determined.

DETD . . . Of these, one was found to be TbRa35, described above, and one was found to be the previously identified M. ***tuberculosis*** antigen, HSP60. Of the remaining eight clones, seven (hereinafter referred to as RDIF2, RDIF5, RDIF8, RDIF10, RDIF11 and RDIF 12) were found to bear some similarity to previously identified M. ***tuberculosis*** sequences. The determined DNA sequences for RDIF2, RDIF5, RDIF8, RDIF10 and RDIF11 are provided in SEQ ID Nos.: 189-193, respectively,. . .

DETD . . . 8A-B and 9A-B, these antigens were found to stimulate cell proliferation and IFN-.gamma. production in T cells isolated from M. ***tuberculosis*** -immune donors.

DETD [0525] An M. ***tuberculosis*** polypeptide was isolated from tuberculin purified protein derivative (PPD) as follows.

DETD . . . F. et al., Tuberculin purified protein derivative. Preparation and analyses of a large quantity for standard. The American Review of ***Tuberculosis*** 44:9-25, 1941).

DETD [0527] M. ***tuberculosis*** Rv strain was grown for 6 weeks in synthetic medium in roller bottles at 37.degree. C. Bottles containing the bacterial. . .

DETD [0528] Six fractions were collected, dried, suspended in PBS and tested individually in M. ***tuberculosis*** -infected guinea pigs for induction of delayed type hypersensitivity (DTH) reaction. One fraction was found to induce a strong DTH reaction. . . 80 .mu.l/minute. Eluent was monitored at 215 nm. Eight fractions were collected and tested for induction of DTH in M. ***tuberculosis*** -infected guinea pigs. One fraction was found to induce strong DTH of about 16 mm induration. The other fractions did not. . .

DETD . . . were isolated and found to have the sequences shown in SEQ ID Nos.: 130-133. A subsequent search of the M. ***tuberculosis*** genome database released by the Institute for Genomic Research revealed a match of the DPPD partial amino acid sequence with a sequence present within the M. ***tuberculosis*** cosmid MTY21C12. An open reading frame of 336 bp was identified. The full-length DNA sequence for DPPD is provided in. . .

DETD Use of Sera From ***Tuberculosis*** -Infected Monkeys to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD [0531] Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Serum samples were obtained from a cynomolgous monkey 18, 33, 51 and 56 days following infection with M. ***tuberculosis*** Erdman strain. These samples were pooled and used to screen the M. ***tuberculosis*** genomic DNA expression library using the procedure described above in Example 3C.

DETD . . . MO-35 were found to show a high degree of relatedness and showed some homology to a previously identified unknown M. ***tuberculosis*** sequence and to cosmid MTC1237. MO-2 was found to

show some homology to aspartokinase from M. ***tuberculosis*** . Clones MO-3, MO-7 and MO-27 were found to be identical and to show a high degree of relatedness to MO-5. All four of these clones showed some homology to M. ***tuberculosis*** heat shock protein 70. MO-27 was found to show some homology to M. ***tuberculosis*** cosmid MTCY339. MO-4 and MO-34 were found to show some homology to cosmid SCY21B4 and M. smegmatis integration host factor, and were both found to show some homology to a previously identified, unknown M. ***tuberculosis*** sequence. MO-6 was found to show some homology to M. ***tuberculosis*** heat shock protein 65. MO-8, MO-9, MO-10, MO-26 and MO-29 were found to be highly related to each other and to show some homology to M. ***tuberculosis*** dihydrolipamide succinyltransferase. MO-28, MO-31 and MO-32 were found to be identical and to show some homology to a previously identified M. ***tuberculosis*** protein. MO-33 was found to show some homology to a previously identified 14 kDa M. ***tuberculosis*** heat shock protein.

DETD . . . homologies to MO-39. MO-12, MO-13 and MO-19 were found to show some homologies to unknown sequences previously isolated from M. ***tuberculosis*** .

DETD Isolation of DNA Sequences Encoding M. ***tuberculosis*** Antigens by Screening of a Novel Expression Library

DETD [0535] This example illustrates isolation of DNA sequences encoding M. ***tuberculosis*** antigens by screening of a novel expression library with sera from M. ***tuberculosis*** -infected patients that were shown to be unreactive with a panel of the recombinant M. ***tuberculosis*** antigens TbRa11, TbRa3, Tb38-1, TbH4, TbF and 38 kD.

DETD [0536] Genomic DNA from M. ***tuberculosis*** Erdman strain was randomly sheared to an average size of 2 kb, and blunt ended with Klenow polymerase, followed by. . . Wis.) and packaged in vitro using the PhageMaker extract (Novagen). The resulting library was screened with sera from several M. ***tuberculosis*** donors that had been shown to be negative on a panel of previously identified M. ***tuberculosis*** antigens as described above in Example 3B.

DETD . . . 330-332, 334, 336, 338, 340 and 342 were found to show some homology to unknown sequences previously identified in M. ***tuberculosis*** .

DETD Isolation of Soluble M. ***tuberculosis*** Antigens Using Mass Spectrometry

DETD [0538] This example illustrates the use of mass spectrometry to identify soluble M. ***tuberculosis*** antigens.

DETD [0539] In a first approach, M. ***tuberculosis*** culture filtrate was screened by Western analysis using serum from a ***tuberculosis*** -infected individual. The reactive bands were excised from a silver stained gel and the amino acid sequences determined by mass spectrometry.. . . of this sequence with those in the gene bank revealed homology to the 85b precursor antigen previously identified in M. ***tuberculosis*** .

DETD [0540] In a second approach, the high molecular weight region of M. ***tuberculosis*** culture supernatant was studied. This area may contain immunodominant antigens which may be useful in the diagnosis of M. ***tuberculosis*** infection. Two known monoclonal antibodies, IT42 and IT57 (available from the Center for Disease Control, Atlanta, Ga.), show reactivity by. . . the antigens remains unknown. In

addition, unknown high-molecular weight proteins have been described as containing a surrogate marker for M. ***tuberculosis*** infection in HIV-positive individuals (Jnl. Infect. Dis., 176:133-143, 1997). To determine the identity of these antigens, two-dimensional gel electrophoresis and. . .

DETD . . . phosphoenolpyruvate kinase. The two sequences isolated from spot 2 were determined to be from two DNAks, previously identified in M. ***tuberculosis*** as heat shock proteins. Spot 4 was determined to be the previously identified M. ***tuberculosis*** protein Kat G. To the best of the inventors' knowledge, neither PcK-1 nor the two DNAks have previously been shown to have utility in the diagnosis of M. ***tuberculosis*** infection.

DETD Use of Representative Antigens for Diagnosis of ***Tuberculosis***

DETD [0542] This example illustrates the effectiveness of several representative polypeptides in skin tests for the diagnosis of M. ***tuberculosis*** infection.

DETD . . . 20 individuals tested, 2 were PPD negative and 18 were PPD positive. Of the PPD positive individuals, 3 had active ***tuberculosis*** , 3 had been previously infected with ***tuberculosis*** and 9 were healthy. In a second study, 13 PPD positive individuals were tested with 0.1 .mu.g TbRa11 in either. . .

DETD Preparation and Characterization of M. ***tuberculosis*** Fusion Proteins

DETD [0555] The reactivity of the fusion protein TbF-2 with sera from M. ***tuberculosis*** -infected patients was examined by ELISA using the protocol described above. The results of these studies (Table 11) demonstrate that all. . .

DETD [0557] Genomic M. ***tuberculosis*** DNA was used to PCR full-length TbH4 (FL TbH4) with the primers PDM-157 and PDM-160 (SEQ ID NO: 348 and. . .

DETD SEQUENCE CHARACTERISTICS:
LENGTH: 53 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 146
GGATCCATAT GGGCCATCAT CATCATCATC ACGTGATCGA CATCATCGGG ACC 53

DETD SEQUENCE CHARACTERISTICS:
LENGTH: 42 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR Primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 147
CCTGAATTCA GGCCTCGGTT GCGCCGGCCT CATCTTGAAC GA 42

DETD SEQUENCE CHARACTERISTICS:
LENGTH: 31 base pairs
TYPE: nucleic acid

STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR Primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 148
GGATCCTGCA GGCTCGAAAC CACCGAGCGG T 31
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 31 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 149
CTCTGAATTC AGCGCTGGAA ATCGTCGCGA T 31
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 33 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 150
GGATCCAGCG CTGAGATGAA GACCGATGCC GCT 33
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 33 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 151
GAGAGAATTC TCAGAAGCCC ATTTGCGAGG ACA 33
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 1993 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: DNA (genomic)
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
FEATURE:
NAME/KEY: CDS
LOCATION: 152..1273
SEQUENCE: 152
TGTTCTTCGA CGGCAGGCTG GTGGAGGAAG GGCCCACCGA ACAGCTGTTC TCCTCGCCGA 60
AGCATGCGGA AACCGCCCGA TACGTCGCCG GACTGTCGGG GGACGTCAAG GACGCCAAGC 120

GCGGAAATTG AAGAGCACAG AAAGGTATGG. . .
CLM What is claimed is:

1. A polypeptide comprising an immunogenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen has an. . .

2. A polypeptide comprising an immunogenic portion of an M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen has an. . .

3. A polypeptide comprising an immunogenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an. . .

4. A polypeptide comprising an immunogenic portion of a M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an. . .

23. A fusion protein comprising one or more polypeptides according to any one of claims 1-4 and the M. ***tuberculosis*** antigen 38 kD (SEQ ID NO: 155).

29. A method for detecting ***tuberculosis*** in a patient, comprising: (a) contacting dermal cells of a patient with one or more polypeptides according to any one of claims 14; and (b) detecting an immune response on the patient's skin and therefrom detecting ***tuberculosis*** in the patient.

30. A method for detecting ***tuberculosis*** in a patient, comprising: (a) contacting dermal cells of a patient with a polypeptide having an N-terminal sequence selected from. . . in SEQ ID NO: 134 and 135; and (b) detecting an immune response on the patient's skin and therefrom detecting ***tuberculosis*** in the patient.

31. A method for detecting ***tuberculosis*** in a patient, comprising: (a) contacting dermal cells of a patient with one or more polypeptides encoded by a DNA. . . 330-332, 334, 336, 338, 340 and 342-347; and (b) detecting an immune response on the patient's skin and therefrom detecting ***tuberculosis*** in the patient.

L4 ANSWER 2 OF 2 USPATFULL on STN
AN 2003:195215 USPATFULL
TI Compounds and methods for diagnosis of ***tuberculosis***
IN ***Reed, Steven G.*** , Bellevue, WA, UNITED STATES
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PA Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)
PI US 2003135026 A1 20030717

AI US 2002-193002 A1 20020710 (10)
RLI Continuation of Ser. No. US 1998-72596, filed on 5 May 1998, GRANTED, Pat. No. US 6458366 Continuation-in-part of Ser. No. US 1998-24753, filed on 18 Feb 1998, ABANDONED Continuation-in-part of Ser. No. US 1997-942341, filed on 1 Oct 1997, ABANDONED Continuation-in-part of Ser. No. US 1997-818111, filed on 13 Mar 1997, GRANTED, Pat. No. US 6338852 Continuation-in-part of Ser. No. US 1996-729622, filed on 11 Oct 1996, ABANDONED A 371 of International Ser. No. WO 1996-US14675, filed on 30 Aug 1996, PENDING A 371 of International Ser. No. US 1996-680574, filed on 12 Jul 1996, ABANDONED Continuation-in-part of Ser. No. US 1996-658800, filed on 5 Jun 1996, ABANDONED Continuation-in-part of Ser. No. US 1996-620280, filed on 22 Mar 1996, ABANDONED Continuation-in-part of Ser. No. US 1995-532136, filed on 22 Sep 1995, ABANDONED Continuation of Ser. No. US 1995-523435, filed on 1 Sep 1995, ABANDONED
DT Utility
FS APPLICATION
LREP TOWNSEND AND TOWNSEND AND CREW, LLP, TWO EMBARCADERO CENTER, EIGHTH FLOOR, SAN FRANCISCO, CA, 94111-3834
CLMN Number of Claims: 54
ECL Exemplary Claim: 1
DRWN 19 Drawing Page(s)
LN.CNT 9455
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Compounds and methods for diagnosing ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of one or more M. ***tuberculosis*** proteins, and DNA sequences encoding such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of M. ***tuberculosis*** infection in patients and biological samples. Antibodies directed against such polypeptides are also provided.
TI Compounds and methods for diagnosis of ***tuberculosis***
IN ***Reed, Steven G.*** , Bellevue, WA, UNITED STATES
AB Compounds and methods for diagnosing ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of one or more M. ***tuberculosis*** proteins, and DNA sequences encoding such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of M. ***tuberculosis*** infection in patients and biological samples. Antibodies directed against such polypeptides are also provided.
SUMM [0002] The present invention relates generally to the detection of Mycobacterium ***tuberculosis*** infection. The invention is more particularly related to polypeptides comprising a Mycobacterium ***tuberculosis*** antigen, or a portion or other variant thereof, and the use of such polypeptides for the serodiagnosis of Mycobacterium ***tuberculosis*** infection.
SUMM [0003] ***Tuberculosis*** is a chronic, infectious disease, that is generally caused by infection with Mycobacterium ***tuberculosis*** . It is a major disease in developing countries, as well as an increasing problem in developed areas of the world,. . .
SUMM [0004] Although ***tuberculosis*** can generally be controlled using extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease.. . .
SUMM [0005] Inhibiting the spread of ***tuberculosis*** will require

effective vaccination and accurate, early diagnosis of the disease. Currently, vaccination with live bacteria is the most efficient. . .

SUMM [0006] While macrophages have been shown to act as the principal effectors of M. ***tuberculosis*** immunity, T cells are the predominant inducers of such immunity. The essential role of T cells in protection against M. ***tuberculosis*** infection is illustrated by the frequent occurrence of M. ***tuberculosis*** in AIDS patients, due to the depletion of CD4 T cells associated with human immunodeficiency virus (HIV) infection. Mycobacterium-reactive CD4. . . that 1,25-dihydroxy-vitamin D3, either alone or in combination with IFN-.gamma. or tumor necrosis factor-alpha, activates human macrophages to inhibit M. ***tuberculosis*** infection. Furthermore, it is known that IFN-.gamma. stimulates human macrophages to make 1,25dihydroxy-vitamin D3. Similarly, IL-12 has been shown to play a role in stimulating resistance to M. ***tuberculosis*** infection. For a review of the immunology of M. ***tuberculosis*** infection see Chan and Kaufmann, in ***Tuberculosis*** : Pathogenesis, Protection and Control, Bloom (ed.), ASM Press, Washington, D.C., 1994.

SUMM [0007] Accordingly, there is a need in the art for improved diagnostic methods for detecting ***tuberculosis*** . The present invention fulfills this need and further provides other related advantages.

SUMM [0008] Briefly stated, the present invention provides compositions and methods for diagnosing ***tuberculosis*** . In one aspect, polypeptides are provided comprising an antigenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. In one embodiment of. . .

SUMM [0010] In a related aspect, polypeptides are provided comprising an immunogenic portion of an M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, the antigen having one. . .

SUMM [0012] In another embodiment, the soluble M. ***tuberculosis*** antigen comprises an amino acid sequence encoded by a DNA sequence selected from the group consisting of the sequences recited. . .

SUMM [0013] In a related aspect, the polypeptides comprise an antioenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, wherein the antigen comprises. . .

SUMM . . . provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, an inventive polypeptide and a known M. ***tuberculosis*** antigen.

SUMM [0016] In further aspects of the subject invention, methods and diagnostic kits are provided for detecting ***tuberculosis*** in a patient The methods comprise: (a) contacting a biological sample with at least one of the above polypeptides; and (b) detecting in the sample the presence of antibodies that bind to the polypeptide or polypeptides, thereby detecting M. ***tuberculosis*** infection in the biological sample. Suitable biological samples include whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine. The. . .

SUMM [0017] The present invention also provides methods for detecting M. ***tuberculosis*** infection comprising: (a) obtaining a biological sample from a patient; (b) contacting the sample with at least one oligonucleotide primer. . .

SUMM [0018] In a further aspect, the present invention provides a method for detecting M. ***tuberculosis*** infection in a patient comprising:

(a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide. . .

SUMM . . . monoclonal, that bind to the polypeptides described above, as well as methods for their use in the detection of M. ***tuberculosis*** infection.

DRWD . . . illustrate the stimulation of proliferation and interferon-.gamma. .gamma. production in T cells derived from a first and a second M. ***tuberculosis*** --immune donor, respectively, by the 14 Kd, 20 Kd and 26 Kd antigens described in Example 1.

DRWD [0022] FIGS. 2A-D illustrate the reactivity of antisera raised against secretory M. ***tuberculosis*** proteins, the known M. ***tuberculosis*** antigen 85b and the inventive antigens Tb38-1 and TbH-9, respectively, with M. ***tuberculosis*** lysate (lane 2), M. ***tuberculosis*** secretory proteins (lane 3), recombinant Tb38-1 (lane 4), recombinant TbH-9 (lane 5) and recombinant 85b (lane 5).

DRWD [0023] FIG. 3A illustrates the stimulation of proliferation in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, recombinant TbH-9 and a control antigen, TbRa11.

DRWD [0024] FIG. 3B illustrates the stimulation of interferon-.gamma. production in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, PPD and recombinant TbH-9.

DRWD [0025] FIG. 4 illustrates the reactivity of two representative polypeptides with sera from M. ***tuberculosis*** --infected and uninfected individuals, as compared to the reactivity of bacterial lysate.

DRWD [0026] FIG. 5 shows the reactivity of four representative polypeptides with sera from M. ***tuberculosis*** -infected and uninfected individuals, as compared to the reactivity of the 38 kD antigen.

DRWD [0027] FIG. 6 shows the reactivity of recombinant 38 kD and ThRa11 antigens with sera from M. ***tuberculosis*** patients, PPD positive donors and normal donors.

DRWD [0029] FIG. 8 shows the reactivity of the antigen of SEQ ID NO: 60 with sera from M. ***tuberculosis*** patients and normal donors.

DRWD [0030] FIG. 9 illustrates the reactivity of the recombinant antigen TbH-29 (SEQ ID NO: 137) with sera from M. ***tuberculosis*** patients, PPD positive donors and normal donors as determined by indirect ELISA.

DRWD [0031] FIG. 10 illustrates the reactivity of the recombinant antigen TbH-33 (SEQ ID NO: 140) with sera from M. ***tuberculosis*** patients and from normal donors, and with a pool of sera from M. ***tuberculosis*** patients, as determined both by direct and indirect ELISA FIG. 11 illustrates the reactivity of increasing concentrations of the recombinant antigen TbH-33 (SEQ ID NO: 140) with sera from M. ***tuberculosis*** patients and from normal donors as determined by ELISA.

DRWD . . . FIGS. 12A-E illustrate the reactivity of the recombinant antigens MO-1, MO-2, MO-4, MO-28 and MO-29, respectively, with sera from M. ***tuberculosis*** patients and from normal donors as determined by ELISA.

DETD [0173] SEQ ID NO: 149 is the DNA sequence of the M. ***tuberculosis*** antigen 38 kD.

DETD [0174] SEQ ID NO: 150 is the amino acid sequence of the M. ***tuberculosis*** antigen 38 kD.

DETD [0355] SEQ ID NO: 338 is the determined amino acid sequence for a M. ***tuberculosis*** 85 b precursor homolog

DETD [0368] As noted above, the present invention is generally directed to no compositions and methods for diagnosing ***tuberculosis*** . The compositions of the subject invention include polypeptides that comprise at least one antigenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. Polypeptides within the scope of the present invention include, but are not limited to, soluble M. ***tuberculosis*** antigens. A "soluble M. ***tuberculosis*** antigen" is a protein of M. ***tuberculosis*** origin that is present in M. ***tuberculosis*** culture filtrate. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e.,. . . entirely of the antigenic portion, or may contain additional sequences. The additional sequences may be derived from the native M. ***tuberculosis*** antigen or may be heterologous, and such sequences may (but need not) be antigenic.

DETD . . . may or may not be soluble) is a portion that is capable of reacting with sera obtained from an M. ***tuberculosis*** -infected individual (i.e., generates an absorbance reading with sera from infected individuals that is at least three standard deviations above the absorbance obtained with sera from uninfected individuals, in a representative ELISA assay described herein). An "M. ***tuberculosis*** -infected individual" is a human who has been infected with M. ***tuberculosis*** (e.g. has an intradermal skin test response to PPD that is at least 0.5 cm in diameter). Infected individuals may display symptoms of ***tuberculosis*** or may be free of disease symptoms. Polypeptides comprising at least an antigenic portion of one or more M. ***tuberculosis*** antigens as described herein may generally be used, alone or in combination, to detect ***tuberculosis*** in a patient.

DETD . . . be identified by evaluating a modified polypeptide for the ability to generate antibodies that detect the presence or absence of ***tuberculosis*** . Such modified sequences may be prepared and tested using, for example, the representative procedures described herein.

DETD . . . polypeptide" is a polypeptide comprising at least one of the above antigenic portions and one or more additional antigenic M. ***tuberculosis*** sequences, which are joined via a peptide linkage into a single amino acid chain. The sequences may be joined directly. .

.

DETD [0375] In general, M. ***tuberculosis*** antigens, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, soluble antigens may be isolated from M. ***tuberculosis*** culture filtrate by procedures known to those of ordinary skill in the art, including anion-exchange and reverse phase chromatography. Purified. . . may then be evaluated for a desired property, such as the ability to react with sera obtained from an M. ***tuberculosis*** -infected individual. Such screens may be performed using the representative methods described herein. Antigens may then be partially sequenced using, for. . .

DETD . . . and 15 expressed in an appropriate host. DNA molecules encoding soluble antigens may be isolated by screening an appropriate M. ***tuberculosis*** expression library with anti-sera (e.g., rabbit) raised specifically against soluble M. ***tuberculosis*** antigens. DNA sequences encoding antigens that may or may not be soluble may be identified by screening an appropriate M. ***tuberculosis*** genomic or cDNA expression library with sera obtained from patients infected

with M. ***tuberculosis*** . Such screens may generally be performed using techniques well known in the art, such as those described in Sambrook et. . .

DETD [0377] DNA sequences encoding soluble antigens may also be obtained by screening an appropriate M. ***tuberculosis*** cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of. . .

DETD . . . antigens described herein are "antigenic." More specifically, the antigens have the ability to react with sera obtained from an M. ***tuberculosis*** -infected individual. Reactivity may be evaluated using, for example, the representative ELISA assays described herein, where an absorbance reading with sera. . .

DETD [0379] Antigenic portions of M. ***tuberculosis*** antigens may be prepared and identified using well known techniques, such as those summarized in Paul, Fundamental Immunology, 3d ed.,. . . is substantially similar to that generated by the full length antigen. In other words, an antigenic portion of a M. ***tuberculosis*** antigen generates at least about 20%, and preferably about 100%, of the signal induced by the full length antigen in. . .

DETD [0380] Portions and other variants of M. ***tuberculosis*** antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally. . .

DETD [0384] In certain specific embodiments, the subject invention discloses polypeptides comprising at least an antigenic portion of a soluble M. ***tuberculosis*** antigen (or a variant of such an antigen), where the antigen has one of the following N-terminal sequences:

(a) Asp-Pro-Val-Asp-Ala-Val-Ile-Asn-Thr-Thr-Cys-Asn-Tyr-Gly-. . .

DETD [0386] In a further specific embodiment, the subject invention discloses polypeptides comprising at least an immunogenic portion of an M. ***tuberculosis*** antigen having one of the following N-terminal sequences, or a variant thereof that differs only in conservative substitutions and/or modifications:. . .

DETD [0388] In other specific embodiments, the subject invention discloses polypeptides comprising at least an antigenic portion of a soluble M. ***tuberculosis*** antigen (or a variant of such an antigen) that comprises one or more of the amino acid sequences encoded by. . .

DETD [0389] In further specific embodiments, the subject invention discloses polypeptides comprising at least an antigenic portion of a M. ***tuberculosis*** antigen (or a variant of such an antigen), which may or may not be soluble, that comprises one or more. . .

DETD . . . comprising a first and a second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known M. ***tuberculosis*** antigen, such as the 38 kD antigen described in Andersen and Hansen, Infect. Immun. 57:2481-2488, 1989, (Genbank Accession No. M30046). . .

DETD [0393] In another aspect, the present invention provides methods for using the polypeptides described above to diagnose ***tuberculosis*** . In this aspect, methods are provided for detecting M. ***tuberculosis*** infection in a biological sample, using one or more of the above polypeptides, alone or in combination. In embodiments in. . . a predetermined cut-off value. The presence of such antibodies indicates previous sensitization to mycobacterial antigens which may be indicative of ***tuberculosis*** .

DETD . . . using each polypeptide individually to evaluate serum samples obtained from a series of patients known to be infected with M. ***tuberculosis*** . After determining which samples test positive (as described below) with each polypeptide, combinations of two or more polypeptides may be. . . infection in most, or all, of the samples tested. Such polypeptides are complementary. For example, approximately 25-30% of sera from ***tuberculosis*** -infected individuals are negative for antibodies to any single protein, such as the 38 kD antigen mentioned above. Complementary polypeptides may,. . .

DETD . . . (i.e., incubation time) is that period of time that is sufficient to detect the presence of antibody within a M. ***tuberculosis*** -infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that. . .

DETD [0403] To determine the presence or absence of anti M. ***tuberculosis*** antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally. . . general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for ***tuberculosis*** . In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of. . . a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for ***tuberculosis*** .

DETD . . . reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of anti-M. ***tuberculosis*** antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line,. . .

DETD [0409] Antibodies may be used in diagnostic tests to detect the presence of M. ***tuberculosis*** antigens using assays similar to those detailed above and other techniques well known to those of skill in the art, thereby providing a method for detecting M. ***tuberculosis*** infection in a patient.

DETD . . . example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify M. ***tuberculosis*** -specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a DNA molecule. . .

DETD . . . art (see, for example, Mullis et al. Ibid; Ehrlich, Ibid). Primers or probes may thus be used to detect M. ***tuberculosis*** -specific sequences in biological samples. DNA probes or primers comprising oligonucleotide sequences described above may be used alone, in combination with. . .

DETD Purification and Characterization of Polypeptides From M. ***tuberculosis*** Culture Filtrate

DETD [0413] This example illustrates the preparation of M. ***tuberculosis*** soluble polypeptides from culture filtrate. Unless otherwise noted, all percentages in the following example are weight per volume.

DETD [0414] M. ***tuberculosis*** (either H37Ra, ATCC No. 25177, or H37Rv, ATCC No. 25618) was cultured in sterile GAS media at 37.degree. C. for. . .

DETD [0426] Additional soluble antigens were isolated from M. ***tuberculosis*** culture filtrate as follows. M.

tuberculosis culture filtrate was prepared as described above. Following dialysis against Bis-Tris propane buffer, at pH 5.5, fractionation was performed using. . .

DETD . . . DNA sequences that encode the antigens designated as (a), (c), (d) and (g) above were obtained by screening a M. ***tuberculosis*** genomic library using .sup.32P end labeled degenerate oligonucleotides corresponding to the N-terminal sequence and containing M. ***tuberculosis*** codon bias. The screen performed using a probe corresponding to antigen (a) above identified a clone having the sequence provided. . .

DETD . . . was amplified from genomic DNA using the sequence obtained from GENBANK. This sequence was then used to screen an M. ***tuberculosis*** library and a fill length copy of the M. ***tuberculosis*** homologue was obtained (SEQ ID NO: 94).

DETD [0434] The amino acid sequence for antigen (j) was found to be homologous to a known M. ***tuberculosis*** protein translated from a DNA sequence. To the best of the inventors' knowledge, this protein has not been previously shown. . .

DETD Use of Patient Sera to Isolate M. ***tuberculosis*** Antigens

DETD [0437] This example illustrates the isolation of antigens from M. ***tuberculosis*** lysate by screening with serum from M. ***tuberculosis*** -infected individuals.

DETD [0438] Dessicated M. ***tuberculosis*** H37Ra (Difco Laboratories) was added to a 2% NP40 solution, and alternately homogenized and sonicated three times. The resulting suspension. . . a Centriprep 10 (Amicon, Beverley, Mass.) and screened by Western blot for serological activity using a serum pool from M. ***tuberculosis*** -infected patients which was not immunoreactive with other antigens of the present invention.

DETD [0441] A DNA sequence that encodes the antigen designated as (m) above was obtained by screening a genomic M. ***tuberculosis*** Erdman strain library using labeled degenerate oligonucleotides corresponding to the N-terminal sequence of SEQ ID NO: 137. A clone was. . . NO: 199. Comparison of these sequences with those in the genebank revealed some similarity to sequences previously identified in M. ***tuberculosis*** and M. bovis.

DETD Preparation of DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD [0442] This example illustrates the preparation of DNA sequences encoding M. ***tuberculosis*** antigens by screening a M. ***tuberculosis*** expression library with sera obtained from patients infected with M. ***tuberculosis*** , or with anti-sera raised against M. ***tuberculosis*** antigens.

DETD [0443] A. Preparation of M. ***tuberculosis*** Soluble Antigens Using Rabbit Anti-Sera Raised Against M. ***tuberculosis*** Supernatant

DETD [0444] Genomic DNA was isolated from the M. ***tuberculosis*** strain H37Ra. The DNA was randomly sheared and used to construct an expression library using the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Rabbit anti-sera was generated against secretory proteins of the M. ***tuberculosis*** strains H37Ra, H37Rv and Erdman by immunizing a rabbit with concentrated supernatant of the M. ***tuberculosis*** cultures. Specifically, the rabbit was first immunized subcutaneously with 200 .mu.g of protein antigen in a total volume of 2. . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the

nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD [0445] Thirty two clones were purified. Of these, 25 represent sequences that have not been previously identified in M. ***tuberculosis*** . Proteins were induced by IPTG and purified by gel elution, as described in Skeiky et al., J. Exp. Med. 181:1527-1537,. . .

DETD . . . ID NOS: 77, 69, 71, 76) show some homology to sequences previously identified in Mycobacterium leprae but not in M. ***tuberculosis*** . TbRA2A was found to be a lipoprotein, with a six residue lipidation sequence being located adjacent to a hydrophobic secretory sequence. TbRA11, TbRA26, TbRA28 and TbDPEP (SEQ ID NOS: 66, 74, 75, 53) have been previously identified in M. ***tuberculosis*** . No significant homologies were found to TbRA1, TbRA3, TbRA4, TbRA9, TbRA10, TbRA13, TbRA17, TbRA19, TbRA29, TbRA32, TbRA36 and the overlapping. . .

DETD B. Use of Sera From Patients Having Pulmonary or Pleural ***Tuberculosis*** to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD . . . DNA library described above, and an additional H37Rv library, were screened using pools of sera obtained from patients with active ***tuberculosis*** . To prepare the H37Rv library, M. ***tuberculosis*** strain H37Rv genomic DNA was isolated, subjected to partial Sau3A digestion and used to construct an expression library using the. . . and TbH=high reactivity) in both ELISA and immunoblot format. A fourth pool of sera from seven patients with active pulmonary ***tuberculosis*** was also employed. All of the sera lacked increased reactivity with the recombinant 38 kDt M. ***tuberculosis*** H37Ra phosphate-binding protein.

DETD . . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD [0449] Thirty two clones were purified. Of these, 31 represented sequences that had not been previously identified in human M. ***tuberculosis*** . Representative sequences of the DNA molecules identified are provided in SEQ ID NOS: 26-51 and 100. Of these, TbH-8-2 (SEQ. . . of the open reading frame for the antigen ESAT-6 previously identified in M. bovis (Acc. No. U34848) and in M. ***tuberculosis*** (Sorensen et al., Infec. Immun. 63:1710-1717. 1995).

DETD . . . sequence of Tb38-1F3 is presented in SEQ. ID. NO. 112. A TbH-9 probe identified three clones in the H37Rv library: ***TbH*** - ***9*** - ***FL*** (SEQ. ID NO. 101), which may be the homologue of TbH-9 (R37Ra), TbH-9-1 (SEQ. ID NO. 103), and TbH-8-2 (SEQ.. . .

DETD [0453] Further screening of the M. ***tuberculosis*** genomic DNA library, as described above, resulted in the recovery of ten additional reactive clones, representing seven different genes. One. . . determined to be identical to the 14 Kd alpha crystallin heat shock protein previously shown to be present in M. ***tuberculosis*** , and a third was determined to be identical to the antigen TbH-8 described above. The determined DNA sequences for the. . . contains the reactive open reading frame), although the 3' end of TbH-29 was found to be identical to the M. ***tuberculosis*** cosmid Y227. TbH-32 and TbH-33 were found to be identical to the previously identified M. ***tuberculosis*** insertion element IS6110 and to the M. ***tuberculosis*** cosmid Y50, respectively. No significant homologies to TbH-30 were found.

DETD . . . and uninduced lysates were run in duplicate on SDS-PAGE and

transferred to nitrocellulose filters. Filters were reacted with human M. ***tuberculosis*** sera (1:200 dilution) reactive with TbH and a rabbit sera (1:200 or 1:250 dilution) reactive with the N-terminal 4 Kd. . .

DETD [0455] Positive reaction of the recombinant human M. ***tuberculosis*** antigens with both the human M. ***tuberculosis*** sera and anti-lacZ sera indicate that reactivity of the human M. ***tuberculosis*** sera is directed towards the fusion protein. Antigens reactive with the anti-lacZ sera but not with the human M. ***tuberculosis*** sera may be the result of the human M. ***tuberculosis*** sera recognizing conformational epitopes, or the antigen-antibody binding kinetics may be such that the 2 hour sera exposure in the. . .

DETD [0456] Studies were undertaken to determine whether the antigens TbH-9 and Tb38-1 represent cellular proteins or are secreted into M. ***tuberculosis*** culture media In the first study, rabbit sera were raised against A) secretory proteins of M. ***tuberculosis*** , B) the known secretory recombinant M. ***tuberculosis*** antigen 85b, C) recombinant Tb38-1 and D) recombinant TbH-9, using protocols substantially as described in Example 3A. Total M. ***tuberculosis*** lysate, concentrated supernatant of M. ***tuberculosis*** cultures and the recombinant antigens 85b, TbH-9 and Tb38-1 were resolved on denaturing gels, immobilized on nitrocellulose membranes and duplicate. . .

DETD . . . FIGS. 2A-D, respectively, wherein the lane designations are as follows: 1) molecular weight protein standards; 2) 5 .mu.g of M. ***tuberculosis*** lysate; 3) 5 .mu.g secretory proteins; 4) 50 ng recombinant Tb38-1; 5) 50 ng recombinant TbH-9; and 6) 50 ng. . . by an arrow). These results demonstrate that Tb38-1 and TbH-9 are intracellular antigens and are not actively secreted by M. ***tuberculosis*** .

DETD . . . an intracellular antigen was confirmed by determining the reactivity of TbH-9-specific human T cell clones to recombinant TbH-9, secretory M. ***tuberculosis*** proteins and PPD. A TbH-9-specific T cell clone (designated 131TbH-9) was generated from PBMC of a healthy PPD-positive donor. The proliferative response of 131TbH-9 to secretory proteins, recombinant TbH-9 and a control M. ***tuberculosis*** antigen, TbRa11, was determined by measuring uptake of tritiated thymidine, as described in Example 1. As shown in FIG. 3A, the clone 131TbH-9 responds specifically to TbH-9, showing that TbH-9 is not a significant component of M. ***tuberculosis*** secretory proteins. FIG. 3B shows the production of IFN-.gamma. by a second TbH-9-specific T cell clone (designated PPD 800-10) prepared. . . cell clone with secretory proteins, PPD or recombinant TbH-9. These results further confirm that TbH-9 is not secreted by M. ***tuberculosis*** .

DETD C. Use of Sera From Patients Having Extrapulmonary ***Tuberculosis*** to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD [0459] Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). The resulting library was screened using pools of sera obtained from individuals with extrapulmonary ***tuberculosis*** , as described above in Example 3B, with the secondary antibody being goat anti-human IgG+A+M (H+L) conjugated with alkaline phosphatase.

DETD . . . the exception of the 3' ends of XP2 and XP6 which were found to

bear some homology to known M. ***tuberculosis*** cosmids. The DNA sequences for XP27 and XP36 are shown in SEQ ID NOS: 158 and 159, respectively, with the. . .

DETD . . . for purification. Recombinant XP1 was found to stimulate cell proliferation and IFN-.gamma. production in T cells isolated from an M. ***tuberculosis*** -immune donors.

DETD D. Use of a Lysate Positive Serum Pool From Patients Having ***Tuberculosis*** to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigenes

DETD [0463] Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda Screen expression system (Novagen, Madison, Wis.), as described below in Example 6. Pooled serum obtained from M. ***tuberculosis*** --infected patients and that was shown to react with M. ***tuberculosis*** lysate but not with the previously expressed proteins 38 kD, Tb38-1, TbRa3. TbH4, DPEP and TbRa11, was used to screen. . .

DETD . . . SEQ ID NO: 252-255. The remaining seventeen clones were found to show similarities to unknown sequences previously identified in M. ***tuberculosis*** . The determined 5' cDNA sequences for sixteen of these clones (hereinafter referred to as LSER-1, LSER-3, LSER-4, LSER-5, LSER-6, LSER-8,. . .

DETD E. Preparation of M. ***tuberculosis*** Soluble Antigens Using Rabbit Anti-Sera Raised Against M. ***tuberculosis*** Fractionated Proteins

DETD [0465] M. ***tuberculosis*** lysate was prepared as described above in Example 2. The resulting material was fractionated by HPLC and the fractions screened by Western blot for serological activity with a serum pool from M. ***tuberculosis*** --infected patients which showed little or no immunoreactivity with other antigens of the present invention. Rabbit anti-sera was generated against the most reactive fraction using the method described in Example 3A. The anti-sera was used to screen an M. ***tuberculosis*** Erdman strain genomic DNA expression library prepared as described above. Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones determined.

DETD . . . Of these, one was found to be TbRa35, described above, and one was found to be the previously identified M. ***tuberculosis*** antigen, HSP60. Of the remaining eight clones, six (hereinafter referred to as RDIF2, RDIF5, RDIF8, RDLF10, RDIF11 and RDIF12) were found to bear some similarity to previously identified M. ***tuberculosis*** sequences. The determined DNA sequences for RDIF2, RDIF5, RDIF8, RDIF10 and RDIF11 are provided in SEQ ID NOS: 184-188, respectively,. . .

DETD . . . as described above. These antigens were found to stimulate cell proliferation and IFN-.gamma. production in T cells isolated from M. ***tuberculosis*** -immune donors.

DETD [0468] An M. ***tuberculosis*** polypeptide was isolated from tuberculin purified protein derivative (PPD) as follows.

DETD . . . F. et al., Tuberculin purified protein derivative. Preparation and analyses of a large quantity for standard. The American Review of ***Tuberculosis*** 44:9-25, 1941). M. ***tuberculosis*** Rv strain was grown for 6 weeks in synthetic medium in roller bottles at 37.degree. C. Bottles containing the bacterial. . .

DETD [0470] Six fractions were collected, dried, suspended in PBS and tested

individually in M. ***tuberculosis*** -infected guinea pigs for induction of delayed type hypersensitivity (DTH) reaction. One fraction was found to induce a strong DTH reaction. . . 80 .mu.l/minute. Eluent was monitored at 215 rm. Eight fractions were collected, and tested for induction of DTH in M. ***tuberculosis*** -infected guinea pigs. One fraction was found to induce strong DTH of about 16 mm induration. The other fractions did not. . .

DETD . . . were isolated and found to have the sequences shown in SEQ ID NOS: 125-128. A subsequent search of the M. ***tuberculosis*** genome database released by the Institute for Genomic Research revealed a match of the DPPD partial amino acid sequence with a sequence present within the M. ***tuberculosis*** cosrid MTY21C12. An open reading frame of 336 bp was identified. The full-length DNA sequence for DPPD is provided in. . .

DETD Use of Sera From ***Tuberculosis*** -Infected Monkeys to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD [0472] Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Serum samples were obtained from a cynomolgous monkey 18, 33, 51 and 56 days following infection with M. ***tuberculosis*** Erdman strain. These samples were pooled and used to screen the M. ***tuberculosis*** genomic DNA expression library using the procedure described above in Example 3C.

DETD . . . MO-35 were found to show a high degree of relatedness and showed some homology to a previously identified unknown M. ***tuberculosis*** sequence and to cosmid MTC1237. MO-2 was found to show some homology to aspartokinase from M. ***tuberculosis*** . Clones MO-3, MO-7 and MO-27 were found to be identical and to show a high degree of relatedness to MO-5. All four of these clones showed some homology to M. ***tuberculosis*** heat shock protein 70. MO-27 was found to show some homology to M. ***tuberculosis*** cosmid MTCY339. MO-4 and MO-34 were found to show some homology to cosmid SCY21B4 and M. smegmatis integration host factor, and were both found to show some homology to a previously identified, unknown M. ***tuberculosis*** sequence. MO-6 was found to show some homology to M. ***tuberculosis*** heat shock protein 65. MO-8, MO-9, MO-10, MO-26 and MO-29 were found to be highly related to each other and to show some homology to M. ***tuberculosis*** dihydrolipamide succinyltransferase. MO-28, MO-31 and MO-32 were found to be identical and to show some homology to a previously identified M. ***tuberculosis*** protein. MO-33 was found to show some homology to a previously identified 14 kDa M. ***tuberculosis*** heat shock protein.

DETD . . . homologies to MO-39. MO-12, MO-13 and MO-19 were found to show some homologies to unknown sequences previously isolated from M. ***tuberculosis*** .

DETD Isolation of DNA Sequences Encoding M. ***tuberculosis*** Antigens by Screening of a Novel Expression Library

DETD [0476] This example illustrates isolation of DNA sequences encoding M. ***tuberculosis*** antigens by screening of a novel expression library with sera from M. ***tuberculosis*** -infected patients that were shown to be unreactive with a panel of the recombinant M. ***tuberculosis*** antigens TbRa11, TbRa3, Tb38-1, TbH4, TbF and 38 kD.

DETD [0477] Genomic DNA from M. ***tuberculosis*** Erdman strain was randomly sheared to an average size of 2 kb, and blunt ended with Kienow polymerase, followed by. . . Wis.) and packaged in vitro using the PhageMaker extract (Novagen). The resulting library was screened with sera from several M. ***tuberculosis*** donors that had been shown to be negative on a panel of previously identified M. ***tuberculosis*** antigens as described above in Example 3B.

DETD . . . 324-326, 328, 330, 332, 334 and 336 were found to show some homology to unknown sequences previously identified in M. ***tuberculosis*** .

DETD Isolation of Soluble M. ***tuberculosis*** Antigens Using Mass Spectrometry

DETD [0479] This example illustrates the use of mass spectrometry to identify soluble M. ***tuberculosis*** antigens.

DETD [0480] In a first approach, M. ***tuberculosis*** culture filtrate was screened by Western analysis using serum from a ***tuberculosis*** -infected individual. The reactive bands were excised from a silver stained gel and the amino acid sequences determined by mass spectrometry.. . . of this sequence with those in the gene bank revealed homology to the 85b precursor antigen previously identified in M. ***tuberculosis*** .

DETD [0481] In a second approach, the high molecular weight region of M. ***tuberculosis*** culture supernatant was studied. This area may contain immunodominant antigens which may be useful in the diagnosis of M. ***tuberculosis*** infection. Two known monoclonal antibodies, IT42 and IT57 (available from the Center for Disease Control, Atlanta, Ga.), show reactivity by. . . the antigens remains unknown. In addition, unknown high-molecular weight proteins have been described as containing a surrogate marker for M. ***tuberculosis*** infection in HIV-positive individuals (Jnl. Infect. Dis.. 1 76:133-143. 1997).

DETD . . . phosphoenolpyruvate kinase. The two sequences isolated from spot 2 were determined to be from two DNAks, previously identified in M. ***tuberculosis*** as heat shock proteins. Spot 4 was determined to be the previously identified M. ***tuberculosis*** protein Kat G. To the best of the inventors' knowledge, neither PcK-1 nor the two DNAks have previously been shown to have utility in the diagnosis of M. ***tuberculosis*** infection.

DETD Use of Representative Antigens for Serodiagnosis of ***Tuberculosis***

DETD . . . ELISA reactivity of two recombinant antigens isolated using method A in Example 3 (TbRa3 and TbRa9) with sera from M. ***tuberculosis*** positive and negative patients. The reactivity of these antigens is compared to that of bacterial lysate isolated from M. ***tuberculosis*** strain H37Ra (Difco, Detroit, Mich.). In both cases, the recombinant antigens differentiated positive from negative sera. Based on cut-off values. . .

DETD [0492] The reactivity of four antigens (TbRa3, TbRa9, TbH4 and TbH12) with sera from a group of M. ***tuberculosis*** infected patients with differing reactivity in the acid fast stain of sputum (Smithwick and David, Tubercle 52:226, 1971) was also examined, and compared to the reactivity of M. ***tuberculosis*** lysate and the 38 kD antigen. The results are presented in Table 3, below:

TABLE 3

REACTIVITY OF ANTIGENS WITH SERA FROM M. ***TUBERCULOSIS*** PATIENTS

Patient	Acid Fast Sputum	ELISA Values Lysate	38 kD	TbRa9	TbH12	TbH4	TbRa3
Tb01B93I-2	++++	1.853	0.634	0.998	1.022	1.030	1.314

Tb01B93I-19. . .

DETD . . . sensitivity of 27 out of 27, indicating that these antigens should complement each other in the serological detection of M. ***tuberculosis*** infection. In addition, several of the recombinant antigens detected positive sera that were not detected using the 38 kD antigen,. . .

DETD [0494] The reactivity of the recombinant antigen TbRa11 with sera from M. ***tuberculosis*** patients shown to be negative for the 38 kD antigen, as well as with sera from PPD positive and normal. . .

DETD . . . After washing, the assay was developed with TMB substrate as described above. The reactivity of TbRa2A with sera from M. ***tuberculosis*** patients and normal donors in shown in Table 4. The mean value for reactivity of TbRa2A with sera from of M. ***tuberculosis*** patients was 0.444 with a standard deviation of 0.309. The mean for reactivity with sera from normal donors was 0.109. . . that the TbRa2A antigen was capable of detecting sera in this category.

TABLE 4

REACTIVITY OF TBRA2A WITH SERA FROM M. ***TUBERCULOSIS*** PATIENTS AND FROM NORMAL DONORS

Serum ID	Status	OD 450
Tb85	TB	0.680
Tb86	TB	0.450
Tb87	TB	0.263
Tb88	TB. . .	

DETD [0496] The reactivity of the recombinant antigen (g) (SEQ ID NO: 60) with sera from M. ***tuberculosis*** patients and normal donors was determined by ELISA as described above. FIG. 8 shows the results of the titration of antigen (g) with four M. ***tuberculosis*** positive sera that were all reactive with the 38 kD antigen and with four donor sera All four positive sera. . .

DETD [0497] The reactivity of the recombinant antigen TbH-29 (SEQ ED NO: 137) with sera from M. ***tuberculosis*** patients, PPD positive donors and normal donors was determined by indirect ELISA as described above. The results are shown in FIG. 9. TbH-29 detected 30 out of 60 M. ***tuberculosis*** sera, 2 out of 8 PPD positive sera and 2 out of 27 normal sera.

DETD . . . results of ELISA tests (both direct and indirect) of the antigen TbH-33 (SEQ ID NO: 140) with sera from M. ***tuberculosis*** patients and from normal donors and with a pool of sera from M. ***tuberculosis*** patients. The mean OD 450 was demonstrated to be higher with sera from M. ***tuberculosis*** patients than from normal donors, with the mean OD 450 being significantly higher in the indirect ELISA than in the direct ELISA. FIG. 11 is a titration curve for the reactivity of recombinant TbH-33 with sera from M. ***tuberculosis*** patients and from normal donors showing an increase

in OD 450 with increasing concentration of antigen.

DETD [0499] The reactivity of the recombinant antigens RDIF6, RDEF8 and RDIF10 (SEQ ID NOS: 184-187, respectively) with sera from M. ***tuberculosis*** patients and normal donors was determined by ELISA as described above. RDIF6 detected 6 out of 32 M. ***tuberculosis*** sera and 0 out of 15 normal sera; RDIF8 detected 14 out of 32 M. ***tuberculosis*** sera and 0 out of 15 normal sera; and RDIF10 detected 4 out of 27 M. ***tuberculosis*** sera and 1 out of 15 normal sera. In addition, RDIF10 was found to detect 0 out of 5 sera. . .

DETD . . . 5, were expressed in E. coli and purified using a hexahistidine tag. The reactivity of these antigens with both M. ***tuberculosis*** positive and negative sera was examined by ELISA as described above. Titration curves showing the reactivity of MO-1, MO-2, MO-4, MO-28 and MO-29 at different solid phase coat levels when tested against four M. ***tuberculosis*** positive sera and four M. ***tuberculosis*** negative sera are shown in FIGS. 12A-E, respectively. Three of the clones, MO-1, MO-2 and MO-29 were further tested on panels of HIV positive/ ***tuberculosis*** (HIV/TB) positive and extrapulmonary sera. MO-1 detected 3/20 extrapulmonary and 2/38 HMV/TB sera. On the same sera groups, MO-2 detected. . . and 16/38 HIV/TB sera. In addition, MO-1 detected 6/17 sera that had previously been shown only to react with M. ***tuberculosis*** lysate and not with either 38 kD or with other antigens of the subject invention.

DETD Preparation and Characterization of M. ***tuberculosis*** Fusion Proteins

DETD [0511] Genomic M. ***tuberculosis*** DNA was used to PCR full-length TbH4 (FL TbH4) with the primers PDM-157 and PDM-160 (SEQ ID NO: 343 and. . .

DETD Use of M. ***tuberculosis*** Fusion Proteins for Serodiagnosis of ***Tuberculosis***

DETD [0515] The effectiveness of the fusion protein TbRa3-38 kD-Tb38-1, prepared as described above, in the serodiagnosis of ***tuberculosis*** infection was examined by ELISA.

DETD . . . 6, with the fusion protein being coated at 200 ng/well. A panel of sera was chosen from a group of ***tuberculosis*** patients previously shown, either by ELISA or by western blot analysis, to react with each of the three antigens individually. . . demonstrates the activity of all three epitopes in the fusion protein.

TABLE 5

REACTIVITY OF TRI-PEPTIDE FUSION PROTEIN WITH SERA FROM M. ***TUBERCULOSIS*** PATIENTS

Serum ID	Status	ELISA and/or Western Blot Reactivity with Individual proteins 38 kd	Fusion Recombinant Tb38-1. . .	Fusion Recombinant

DETD [0517] The reactivity of the fusion protein TbF-2 with sera from M. ***tuberculosis*** -infected patients was examined by ELISA using the protocol described above. The results of these studies (Table 6) demonstrate that all. . .

DETD SEQUENCE CHARACTERISTICS:

LENGTH: 53 base pairs

TYPE: nucleic acid

STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 141
GGATCCATAT GGGCCATCAT CATCATCATC ACGTGATCGA CATCATCGGG ACC 53
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 42 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR Primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 142
CCTGAATTCA GGCCTCGGTT GCGCCGGCCT CATCTTGAAC GA 42
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 31 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR Primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 143
GGATCCTGCA GGCTCGAAAC CACCGAGCGG T 31
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 31 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 144
CTCTGAATTC AGCGCTGGAA ATCGTCGCGA T 31
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 33 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 145
GGATCCAGCG CTGAGATGAA GACCGATGCC GCT 33
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 33 base pairs
TYPE: nucleic acid

STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 146
GAGAGAATTC TCAGAAGCCC ATTTGCGAGG ACA 33
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 1993 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: DNA (genomic)
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
FEATURE:
NAME/KEY: CDS
LOCATION: 152..1273
SEQUENCE: 147
TGTTCTTCGA CGGCAGGCTG GTGGAGGAAG GGCCCACCGA ACAGCTGTTC TCCTCGCCGA 60
AGCATGCGGA AACCGCCCGA TACGTCGCCG GACTGTCGGG GGACGTCAAG GACGCCAAGC 120
GCGGAAATTG AAGAGCACAG AAAGGTATGG. . .
CLM What is claimed is:

1. A polypeptide comprising an antigenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen has an. . .

2. A polypeptide comprising an immunogenic portion of an M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen has an. . .

3. A polypeptide comprising an antigenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an. . .

4. A polypeptide comprising an antigenic portion of a M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an. . .

9. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting a biological sample with one or more polypeptides according to any of. . . detecting in the sample the presence of antibodies that bind to at least one of the polypeptides, thereby detecting M. ***tuberculosis*** infection in the biological sample.

10. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting a biological sample with a polypeptide having an N-terminal sequence selected from. . . detecting in the sample the presence of antibodies that bind to at least one of the polypeptides, thereby detecting M. ***tuberculosis*** infection in the biological sample.

11. A method for detecting M. ***tuberculosis*** infection in a

biological sample, comprising: (a) contacting a biological sample with one or more polypeptides encoded by a DNA. . . detecting in the sample the presence of antibodies that bind to at least one of the polypeptides, thereby detecting M. ***tuberculosis*** infection in the biological sample.

. . . of any one of claims 9-11 wherein step (a) additionally comprises contacting the biological sample with a 38 kD M. ***tuberculosis*** antigen and step (b) additionally comprises detecting in the sample the presence of antibodies that bind to the 38 kD M. ***tuberculosis*** antigen.

17. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting the sample with at least two oligonucleotide primers in a polymerase chain. . . (b) detecting in the sample a DNA sequence that amplifies in the presence of the oligonucleotide primers, thereby detecting M. ***tuberculosis*** infection.

19. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting the sample with at least two oligonucleotide primers in a polymerase chain. . . the sample a DNA sequence that amplifies in the presence of the first and second oligonucleotide primers, thereby detecting M. ***tuberculosis*** infection.

22. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting the sample with one or more oligonucleotide probes specific for a DNA. . . claim 5; and (b) detecting in the sample a DNA sequence that hybridizes to the oligonucleotide probe, thereby detecting M. ***tuberculosis*** infection.

24. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting the sample with one or more oligonucleotide probes specific for a DNA. . . and 337; and (b) detecting in the sample a DNA sequence that hybridizes to the oligonucleotide probe, thereby detecting M. ***tuberculosis*** infection.

27. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting the biological sample with a binding agent which is capable of binding. . . 1-4; and (b) detecting in the sample a protein or polypeptide that binds to the binding agent, thereby detecting M. ***tuberculosis*** infection in the biological sample.

28. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting the biological sample with a binding agent which is capable of binding. . . 130; and (b) detecting in the sample a protein or polypeptide that binds to the binding agent, thereby detecting M. ***tuberculosis*** infection in the biological sample.

29. A method for detecting M. ***tuberculosis*** infection in a

biological sample, comprising: (a) contacting the biological sample with a binding agent which is capable of binding. . . 337; and (b) detecting in the sample a protein or polypeptide that binds to the binding agent, thereby detecting M. ***tuberculosis*** infection in the biological sample.

53. A fusion protein comprising one or more polypeptides according to any one of claims 1-4 and the M. ***tuberculosis*** antigen 38 kD (SEQ ID NO: 150).

=> e skeiky yasir a w/au
E1 16 SKEIKY YASIR/AU
E2 13 SKEIKY YASIR A/AU
E3 228 --> SKEIKY YASIR A W/AU
E4 1 SKEIKY YASIR AW/AU
E5 1 SKEIKY YASSIR A/AU
E6 4 SKEIL D/AU
E7 8 SKEIL D A/AU
E8 6 SKEIL D D/AU
E9 2 SKEIM P/AU
E10 1 SKEIN E V/AU
E11 3 SKEIN W/AU
E12 2 SKEIN WILLEM/AU

=> s e1-e5 and tuberculosis
L5 117 ("SKEIKY YASIR"/AU OR "SKEIKY YASIR A"/AU OR "SKEIKY YASIR A W"/AU OR "SKEIKY YASIR AW"/AU OR "SKEIKY YASSIR A"/AU) AND TUBER CULOSIS

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6 89 DUP REM L5 (28 DUPLICATES REMOVED)

=> s l6 and (TbH-9-fl)
L7 2 L6 AND (TBH-9-FL)

=> d 1-
YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y

L7 ANSWER 1 OF 2 USPATFULL on STN
AN 2003:206886 USPATFULL
TI Compounds and methods for immunotherapy and diagnosis of ***tuberculosis***
IN Reed, Steven G., Bellevue, WA, UNITED STATES
Skeiky, Yasir A.W. , Seattle, WA, UNITED STATES
Dillon, Davin C., Redmond, WA, UNITED STATES
Campos-Neto, Antonio, Bainbridge Island, WA, UNITED STATES
Houghton, Raymond, Bothell, WA, UNITED STATES
Vedvick, Thomas S., Federal Way, WA, UNITED STATES
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Lodes, Michael J., Seattle, WA, UNITED STATES
Hendrickson, Ronald C., Seattle, WA, UNITED STATES
PA Corixa Corporation, Seattle, WA (U.S. corporation)

PI US 2003143243 A1 20030731
AI US 2002-84843 A1 20020225 (10)
RLI Continuation of Ser. No. US 1998-72967, filed on 5 May 1998, PENDING Continuation-in-part of Ser. No. US 1998-25197, filed on 18 Feb 1998, ABANDONED Continuation-in-part of Ser. No. US 1997-942578, filed on 1 Oct 1997, ABANDONED Continuation-in-part of Ser. No. US 1997-818112, filed on 13 Mar 1997, GRANTED, Pat. No. US 6290969 Continuation-in-part of Ser. No. US 1996-730510, filed on 11 Oct 1996, ABANDONED Continuation-in-part of Ser. No. US 1996-680574, filed on 12 Jul 1996, ABANDONED Continuation-in-part of Ser. No. US 1996-659683, filed on 5 Jun 1996, ABANDONED Continuation-in-part of Ser. No. US 1996-620874, filed on 22 Mar 1996, ABANDONED Continuation-in-part of Ser. No. US 1995-533634, filed on 22 Sep 1995, ABANDONED Continuation-in-part of Ser. No. US 1995-523436, filed on 1 Sep 1995, ABANDONED
PRAI WO 1996-US14674 19960830
DT Utility
FS APPLICATION
LN.CNT 9257
INCL INCLM: 424/190.100
INCLS: 530/350.000
NCL NCLM: 424/190.100
NCLS: 530/350.000
IC [7]
ICM: A61K039-015
ICS: A61K039-02; C07K014-35
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 2 OF 2 USPATFULL on STN
AN 2003:195215 USPATFULL
TI Compounds and methods for diagnosis of ***tuberculosis***
IN Reed, Steven G., Bellevue, WA, UNITED STATES
Skeiky, Yasir A.W. , Seattle, WA, UNITED STATES
Dillon, Davin C., Redmond, WA, UNITED STATES
Campos-Neto, Antonio, Bainbridge Island, WA, UNITED STATES
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Vedvick, Thomas S., Federal Way, WA, UNITED STATES
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Lodes, Michael J., Seattle, WA, UNITED STATES
Hendrickson, Ronald C., Seattle, WA, UNITED STATES
PA Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)
PI US 2003135026 A1 20030717
AI US 2002-193002 A1 20020710 (10)
RLI Continuation of Ser. No. US 1998-72596, filed on 5 May 1998, GRANTED, Pat. No. US 6458366 Continuation-in-part of Ser. No. US 1998-24753, filed on 18 Feb 1998, ABANDONED Continuation-in-part of Ser. No. US 1997-942341, filed on 1 Oct 1997, ABANDONED Continuation-in-part of Ser. No. US 1997-818111, filed on 13 Mar 1997, GRANTED, Pat. No. US 6338852 Continuation-in-part of Ser. No. US 1996-729622, filed on 11 Oct 1996, ABANDONED A 371 of International Ser. No. WO 1996-US14675, filed on 30 Aug 1996, PENDING A 371 of International Ser. No. US 1996-680574, filed on 12 Jul 1996, ABANDONED Continuation-in-part of Ser. No. US 1996-658800, filed on 5 Jun 1996, ABANDONED Continuation-in-part of Ser. No. US 1996-620280, filed on 22 Mar 1996, ABANDONED Continuation-in-part of Ser. No. US 1995-532136, filed on 22 Sep 1995, ABANDONED Continuation of Ser. No. US 1995-523435, filed on 1 Sep 1995, ABANDONED

DT Utility
FS APPLICATION
LN.CNT 9455
INCL INCLM: 530/350.000
NCL NCLM: 530/350.000
IC [7]
ICM: C07K001-00
ICS: C07K014-00; C07K017-00
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> e dillon davin c/au
E1 1 DILLON DAVID S/AU
E2 7 DILLON DAVIN/AU
E3 229 --> DILLON DAVIN C/AU
E4 2 DILLON DAVIN CLIFFORD/AU
E5 23 DILLON DEBORAH/AU
E6 12 DILLON DEBORAH A/AU
E7 9 DILLON DEBORAH L/AU
E8 3 DILLON DEBRA W/AU
E9 2 DILLON DECEASED JOHN B/AU
E10 1 DILLON DECEASED JOHN BRADLY/AU
E11 10 DILLON DEIRDRE A/AU
E12 1 DILLON DENNIS/AU

=> s e2-e4 and tuberculosis
L8 112 ("DILLON DAVIN"/AU OR "DILLON DAVIN C"/AU OR "DILLON DAVIN CLIFF ORD"/AU) AND TUBERCULOSIS

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9 93 DUP REM L8 (19 DUPLICATES REMOVED)

=> s l9 and (TbH-9-fl)
L10 2 L9 AND (TBH-9-FL)

=> d 1-
YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y

L10 ANSWER 1 OF 2 USPATFULL on STN
AN 2003:206886 USPATFULL
TI Compounds and methods for immunotherapy and diagnosis of ***tuberculosis***
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PA Corixa Corporation, Seattle, WA (U.S. corporation)
PI US 2003143243 A1 20030731
AI US 2002-84843 A1 20020225 (10)

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PRAI WO 1996-US14674 19960830
DT Utility
FS APPLICATION
LN.CNT 9257
INCL INCLM: 424/190.100
INCLS: 530/350.000
NCL NCLM: 424/190.100
NCLS: 530/350.000
IC [7]
ICM: A61K039-015
ICS: A61K039-02; C07K014-35
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L10 ANSWER 2 OF 2 USPATFULL on STN
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DT Utility
FS APPLICATION

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INCL INCLM: 530/350.000
NCL NCLM: 530/350.000
IC [7]
ICM: C07K001-00
ICS: C07K014-00; C07K017-00
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> e campos neto antonio/au
E1 1 CAMPOS NETO A */AU
E2 1 CAMPOS NETO A C/AU
E3 124 --> CAMPOS NETO ANTONIO/AU
E4 1 CAMPOS NETO C/AU
E5 1 CAMPOS NETO C DE M/AU
E6 1 CAMPOS NETO C M/AU
E7 1 CAMPOS NETO CANTIDIO DE MOURA/AU
E8 4 CAMPOS NETO H M/AU
E9 1 CAMPOS NETO J DE S/AU
E10 1 CAMPOS NETO J M/AU
E11 1 CAMPOS NETO J P/AU
E12 3 CAMPOS NETO J S/AU

=> s e1-e3 and tuberculosis
L11 61 ("CAMPOS NETO A *"/AU OR "CAMPOS NETO A C"/AU OR "CAMPOS NETO ANTONIO"/AU) AND TUBERCULOSIS

=> dup rem l11
PROCESSING COMPLETED FOR L11
L12 45 DUP REM L11 (16 DUPLICATES REMOVED)

=> s l12 and (TbH-9-fl)
L13 2 L12 AND (TBH-9-FL)

=> d 1-
YOU HAVE REQUESTED DATA FROM 2 ANSWERS - CONTINUE? Y/(N):y

L13 ANSWER 1 OF 2 USPATFULL on STN
AN 2003:206886 USPATFULL
TI Compounds and methods for immunotherapy and diagnosis of ***tuberculosis***
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PI US 2003143243 A1 20030731
AI US 2002-84843 A1 20020225 (10)
RLI Continuation of Ser. No. US 1998-72967, filed on 5 May 1998, PENDING
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ABANDONED Continuation-in-part of Ser. No. US 1997-942578, filed on 1 Oct 1997, ABANDONED Continuation-in-part of Ser. No. US 1997-818112, filed on 13 Mar 1997, GRANTED, Pat. No. US 6290969 Continuation-in-part of Ser. No. US 1996-730510, filed on 11 Oct 1996, ABANDONED Continuation-in-part of Ser. No. US 1996-680574, filed on 12 Jul 1996, ABANDONED Continuation-in-part of Ser. No. US 1996-659683, filed on 5 Jun 1996, ABANDONED Continuation-in-part of Ser. No. US 1996-620874, filed on 22 Mar 1996, ABANDONED Continuation-in-part of Ser. No. US 1995-533634, filed on 22 Sep 1995, ABANDONED Continuation-in-part of Ser. No. US 1995-523436, filed on 1 Sep 1995, ABANDONED

PRAI WO 1996-US14674 19960830

DT Utility

FS APPLICATION

LN.CNT 9257

INCL INCLM: 424/190.100
INCLS: 530/350.000

NCL NCLM: 424/190.100
NCLS: 530/350.000

IC [7]
ICM: A61K039-015
ICS: A61K039-02; C07K014-35

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L13 ANSWER 2 OF 2 USPATFULL on STN

AN 2003:195215 USPATFULL

TI Compounds and methods for diagnosis of ***tuberculosis***

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PA Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)

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DT Utility

FS APPLICATION

LN.CNT 9455

INCL INCLM: 530/350.000

NCL NCLM: 530/350.000
IC [7]
ICM: C07K001-00
ICS: C07K014-00; C07K017-00
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> s tuberculosis and (TbH-9-fl)
L14 7 TUBERCULOSIS AND (TBH-9-FL)

=> dup rem l14
PROCESSING COMPLETED FOR L14
L15 7 DUP REM L14 (0 DUPLICATES REMOVED)

=> d bib ab kwic 1-
YOU HAVE REQUESTED DATA FROM 7 ANSWERS - CONTINUE? Y/(N):y

L15 ANSWER 1 OF 7 USPATFULL on STN
AN 2003:206886 USPATFULL
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PRAI WO 1996-US14674 19960830
DT Utility
FS APPLICATION
LREP TOWNSEND AND TOWNSEND AND CREW, LLP, TWO EMBARCADERO CENTER, EIGHTH FLOOR, SAN FRANCISCO, CA, 94111-3834
CLMN Number of Claims: 37
ECL Exemplary Claim: 1
DRWN 19 Drawing Page(s)
LN.CNT 9257
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compounds and methods for inducing protective immunity against ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one immunogenic portion of one or more M. ***tuberculosis*** proteins and DNA molecules encoding such polypeptides. Such compounds may be formulated into vaccines and/or pharmaceutical compositions for immunization against M. ***tuberculosis*** infection, or may be used for the diagnosis of ***tuberculosis*** .

TI Compounds and methods for immunotherapy and diagnosis of ***tuberculosis***

AB Compounds and methods for inducing protective immunity against ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one immunogenic portion of one or more M. ***tuberculosis*** proteins and DNA molecules encoding such polypeptides. Such compounds may be formulated into vaccines and/or pharmaceutical compositions for immunization against M. ***tuberculosis*** infection, or may be used for the diagnosis of ***tuberculosis*** .

SUMM [0002] The present invention relates generally to detecting, treating and preventing Mycobacterium ***tuberculosis*** infection. The invention is more particularly related to polypeptides comprising a Mycobacterium ***tuberculosis*** antigen, or a portion or other variant thereof, and the use of such polypeptides for diagnosing and vaccinating against Mycobacterium ***tuberculosis*** infection.

SUMM [0003] ***Tuberculosis*** is a chronic, infectious disease, that is generally caused by infection with Mycobacterium ***tuberculosis*** . It is a major disease in developing countries, as well as an increasing problem in developed areas of the world,. . .

SUMM [0004] Although ***tuberculosis*** can generally be controlled using extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease.. . .

SUMM [0005] Inhibiting the spread of ***tuberculosis*** requires effective vaccination and accurate, early diagnosis of the disease. Currently, vaccination with live bacteria is the most efficient method. . .

SUMM [0006] While macrophages have been shown to act as the principal effectors of M ***tuberculosis*** immunity, T cells are the predominant inducers of such immunity. The essential role of T cells in protection against M. ***tuberculosis*** infection is illustrated by the frequent occurrence of M. ***tuberculosis*** in AIDS patients, due to the depletion of CD4 T cells associated with human immunodeficiency virus (HIV) infection. Mycobacterium-reactive CD4. . . that 1,25-dihydroxy-vitamin D3, either alone or in combination with IFN-.gamma. or tumor necrosis factor-alpha, activates human macrophages to inhibit M. ***tuberculosis*** infection. Furthermore, it is known that IFN-.gamma. stimulates human macrophages to make 1,25-dihydroxy-vitamin D3. Similarly, IL-12 has been shown to play a role in stimulating resistance to M. ***tuberculosis*** infection. For a review of the immunology of M. ***tuberculosis*** infection see Chan and Kaufmann in ***Tuberculosis*** : Pathogenesis, Protection and Control, Bloom (ed.), ASM Press, Washington, D.C., 1994.

SUMM [0007] Accordingly, there is a need in the art for improved vaccines and methods for preventing, treating and detecting ***tuberculosis*** . The present invention fulfills these needs and further provides other related advantages.

SUMM [0008] Briefly stated, this invention provides compounds and methods for preventing and diagnosing ***tuberculosis*** . In one aspect, polypeptides are provided comprising an immunogenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. In one embodiment of. . .

SUMM [0022] In a related aspect, polypeptides are provided comprising an immunogenic portion of an M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, the antigen having one. . .

SUMM [0026] In another embodiment, the soluble M. ***tuberculosis*** antigen comprises an amino acid sequence encoded by a DNA sequence selected from the group consisting of the sequences recited. . .

SUMM [0027] In a related aspect, the polypeptides comprise an immunogenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, wherein the antigen comprises. . .

SUMM . . . provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, an inventive polypeptide and a known M. ***tuberculosis*** antigen.

SUMM [0032] In further aspects of this invention, methods and diagnostic kits are provided for detecting ***tuberculosis*** in a patient. The methods comprise contacting dermal cells of a patient with one or more of the above polypeptides. . .

SUMM [0033] In yet other aspects, methods are provided for detecting ***tuberculosis*** in a patient, such methods comprising contacting dermal cells of a patient with one or more polypeptides encoded by a. . .

DRWD . . . B illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a first and a second M. ***tuberculosis*** -immune donor, respectively, by the 14 Kd, 20 Kd and 26 Kd antigens described in Example 1.

DRWD [0036] FIG. 2 illustrates the stimulation of proliferation and interferon-.gamma. production in T cells derived from an M. ***tuberculosis*** -immune individual by the two representative polypeptides TbRa3 and TbRa9.

DRWD [0037] FIGS. 3A-D illustrate the reactivity of antisera raised against secretory M. ***tuberculosis*** proteins, the known M. ***tuberculosis*** antigen 85b and the inventive antigens Tb38-1 and TbH-9, respectively, with M. ***tuberculosis*** lysate (lane 2), M. ***tuberculosis*** secretory proteins (lane 3), recombinant Tb38-1 (lane 4), recombinant TbH-9 (lane 5) and recombinant 85b (lane 5).

DRWD [0038] FIG. 4A illustrates the stimulation of proliferation in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, recombinant TbH-9 and a control antigen, TbRa11.

DRWD [0039] FIG. 4B illustrates the stimulation of interferon-.gamma. production in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, PPD and recombinant TbH-9.

DRWD . . . FIGS. 8A and B illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a first M. ***tuberculosis*** -immune individual by the representative polypeptides XP-1, RDIF6, RDIF8, RDIF10 and RDIF11.

DRWD . . . FIGS. 9A and B illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a second M. ***tuberculosis*** -immune individual by the representative

polypeptides XP-1, RDIF6, RDIF8, RDIF10 and RDIF11.

DETD [0190] SEQ ID NO: 154 is the DNA sequence of the M. ***tuberculosis*** antigen 38 kD.

DETD [0191] SEQ ID NO: 155 is the amino acid sequence of the M. ***tuberculosis*** antigen 38 kD.

DETD [0373] SEQ ID NO: 343 is the determined amino acid sequence for a M. ***tuberculosis*** 85b precursor homolog

DETD [0386] As noted above, the present invention is generally directed to compositions and methods for preventing, treating and diagnosing ***tuberculosis*** . The compositions of the subject invention include polypeptides that comprise at least one immunogenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. Polypeptides within the scope of the present invention include, but are not limited to, immunogenic soluble M. ***tuberculosis*** antigens. A "soluble M. ***tuberculosis*** antigen" is a protein of M. ***tuberculosis*** origin that is present in M. ***tuberculosis*** culture filtrate. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e.,. . . . entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native M. ***tuberculosis*** antigen or may be heterologous, and such sequences may (but need not) be immunogenic.

DETD . . . from the group of T cells, NK cells, B cells and macrophages, where the cells are derived from an M. ***tuberculosis*** -immune individual. Polypeptides comprising at least an immunogenic portion of one or more M. ***tuberculosis*** antigens may generally be used to detect ***tuberculosis*** or to induce protective immunity against ***tuberculosis*** in a patient.

DETD . . . be identified by evaluating a modified polypeptide for the ability to generate antibodies that detect the presence or absence of ***tuberculosis*** . Such modified sequences may be prepared and tested using, for example, the representative procedures described herein.

DETD . . . polypeptide" is a polypeptide comprising at least one of the above immunogenic portions and one or more additional immunogenic M. ***tuberculosis*** sequences, which are joined via a peptide linkage into a single amino acid chain. The sequences may be joined directly. . .

DETD [0393] In general, M. ***tuberculosis*** antigens, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, soluble antigens may be isolated from M. ***tuberculosis*** culture filtrate by procedures known to those of ordinary skill in the art, including anion-exchange and reverse phase chromatography. Purified. . .

DETD . . . vector and expressed in an appropriate host. DNA molecules encoding soluble antigens may be isolated by screening an appropriate M. ***tuberculosis*** expression library with anti-sera (e.g., rabbit) raised specifically against soluble M. ***tuberculosis*** antigens. DNA sequences encoding antigens that may or may not be soluble may be identified by screening an appropriate M. ***tuberculosis*** genomic or cDNA expression library with sera obtained from patients infected with M. ***tuberculosis*** . Such screens may generally be performed using techniques well known to those of ordinary skill in the art, such as. . .

DETD [0395] DNA sequences encoding soluble antigens may also be obtained by

screening an appropriate M. ***tuberculosis*** cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of. . .

DETD [0396] Alternatively, genomic or cDNA libraries derived from M. ***tuberculosis*** may be screened directly using peripheral blood mononuclear cells (PBMCs) or T cell lines or clones derived from one or more M. ***tuberculosis*** -immune individuals. In general, PBMCs and/or T cells for use in such screens may be prepared as described below. Direct library. . . of expressed recombinant proteins for the ability to induce proliferation and/or interferon-.gamma. production in T cells derived from an M. ***tuberculosis*** -immune individual. Alternatively, potential T cell antigens may be first selected based on antibody reactivity, as described above.

DETD . . . cytokine production (ie., interferon-.gamma. and/or interleukin-12 production) in T cells, NK cells, B cells and/or macrophages derived from an M. ***tuberculosis*** -immune individual. The selection of cell type for use in evaluating an immunogenic response to a antigen will, of course, depend. . . the desired response. For example, interleukin-12 production is most readily evaluated using preparations containing B cells and/or macrophages. An M. ***tuberculosis*** -immune individual is one who is considered to be resistant to the development of ***tuberculosis*** by virtue of having mounted an effective T cell response to M. ***tuberculosis*** (i.e., substantially free of disease symptoms). Such individuals may be identified based on a strongly positive (i.e., greater than about 10 mm diameter induration) intradermal skin test response to ***tuberculosis*** proteins (PPD) and an absence of any signs or symptoms of ***tuberculosis*** disease. T cells, NK cells, B cells and macrophages derived from M. ***tuberculosis*** -immune individuals may be prepared using methods known to those of ordinary skill in the art. For example, a preparation of. . . individual mycobacterial proteins, may be employed. Such T cell clones may be generated by, for example, culturing PBMCs from M. ***tuberculosis*** -immune individuals with mycobacterial proteins for a period of 2-4 weeks. This allows expansion of only the mycobacterial protein-specific T cells,. . . (i.e., interferon-.gamma. and/or interleukin- 12 production) performed using T cells, NK cells, B cells and/or macrophages derived from an M. ***tuberculosis*** -immune individual are considered immunogenic. Such assays may be performed, for example, using the representative procedures described below. Immunogenic portions of. . .

DETD . . . and/or interleukin-12 production) in T cells, NK cells, B cells and/or macrophages derived from at least about 25% of M. ***tuberculosis*** -immune individuals. Among these immunogenic antigens, polypeptides having superior therapeutic properties may be distinguished based on the magnitude of the responses. . . proliferation and/or cytokine production in vitro in cells derived from more than about 25% of individuals that are not M. ***tuberculosis*** -immune, thereby eliminating responses that are not specifically due to M. ***tuberculosis*** -responsive cells. Those antigens that induce a response in a high percentage of T cell, NK cell, B cell and/or macrophage preparations from M. ***tuberculosis*** -immune individuals (with a low incidence of responses in cell preparations from other individuals) have superior therapeutic properties.

DETD [0401] Antigens with superior therapeutic properties may also be

identified based on their ability to diminish the severity of M. ***tuberculosis*** infection in experimental animals, when administered as a vaccine. Suitable vaccine preparations for use on experimental animals are described in. . .

DETD . . . identified based on the ability to elicit a response in an intradermal skin test performed on an individual with active ***tuberculosis*** , but not in a test performed on an individual who is not infected with M. ***tuberculosis*** . Skin tests may generally be performed as described below, with a response of at least 5 mm induration considered positive.

DETD [0404] Portions and other variants of M. ***tuberculosis*** antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally. . .

DETD [0408] In certain specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a soluble M. ***tuberculosis*** antigen having one of the following N-terminal sequences, or a variant thereof that differs only in conservative substitutions and/or modifications:

DETD [0422] In a further specific embodiment, the subject invention discloses polypeptides comprising at least an immunogenic portion of an M. ***tuberculosis*** antigen having one of the following N-terminal sequences, or a variant thereof that differs only in conservative substitutions and/or modifications:

DETD [0426] In other specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a soluble M. ***tuberculosis*** antigen (or a variant of such an antigen) that comprises one or more of the amino acid sequences encoded by. . .

DETD [0427] In further specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a M. ***tuberculosis*** antigen (or a variant of such an antigen), which may or may not be soluble, that comprises one or more. . .

DETD [0428] In the specific embodiments discussed above, the M. ***tuberculosis*** antigens include variants that are encoded by DNA sequences which are substantially homologous to one or more of DNA sequences. . .

DETD . . . comprising a first and a second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known M. ***tuberculosis*** antigen, such as the 38 kD antigen described in Andersen and Hansen, Infect. Immun. 57:2481-2488, 1989, (Genbank Accession No. M30046). . .

DETD . . . or more of the above polypeptides or fusion proteins (or DNA molecules encoding such polypeptides) to induce protective immunity against ***tuberculosis*** in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be. . . may be free of detectable disease and/or infection. In other words, protective immunity may be induced to prevent or treat ***tuberculosis*** .

DETD . . . adjuvant or a liposome (into which the polypeptide is incorporated). Such pharmaceutical compositions and vaccines may also contain other M. ***tuberculosis*** antigens, either incorporated into a combination polypeptide or present within a separate polypeptide.

DETD . . . above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known M. ***tuberculosis*** antigen, such as the 38 kD antigen described above.

For example, administration of DNA encoding a polypeptide of the present. . .

DETD . . . described above, is capable of raising an immune response in an immunized patient sufficient to protect the patient from M. ***tuberculosis*** infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced in situ. . .

DETD . . . aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune responses, such as lipid A, Bortadella pertussis or Mycobacterium ***tuberculosis*** . Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Freund's Complete Adjuvant (Difco Laboratories) and Merck Adjuvant. . .

DETD [0440] In another aspect, this invention provides methods for using one or more of the polypeptides described above to diagnose ***tuberculosis*** using a skin test. As used herein, a "skin test" is any assay performed directly on a patient in which. . .

DETD . . . than about 0.5 cm in diameter, preferably greater than about 1.0 cm in diameter, is a positive response, indicative of ***tuberculosis*** infection, which may or may not be manifested as an active disease.

DETD Purification and Characterization of Polypeptides From M. ***tuberculosis*** Culture Filtrate

DETD [0445] This example illustrates the preparation of M. ***tuberculosis*** soluble polypeptides from culture filtrate. Unless otherwise noted, all percentages in the following example are weight per volume.

DETD [0446] M. ***tuberculosis*** (either H37Ra, ATCC No. 25177, or H37Rv, ATCC No. 25618) was cultured in sterile GAS media at 37.degree. C. for. . .

DETD [0467] Additional soluble antigens were isolated from M. ***tuberculosis*** culture filtrate as follows. M. ***tuberculosis*** culture filtrate was prepared as described above. Following dialysis against Bis-Tris propane buffer, at pH 5.5, fractionation was performed using. . .

DETD . . . sequences that encode the antigens designated as (a), (c), (d) and (g) above were obtained by screening a genomic M. ***tuberculosis*** library using .sup.32P end labeled degenerate oligonucleotides corresponding to the N-terminal sequence and containing M. ***tuberculosis*** codon bias. The screen performed using a probe corresponding to antigen (a) above identified a clone having the sequence provided. . .

DETD . . . was amplified from genomic DNA using the sequence obtained from GENBANK. This sequence was then used to screen the M. ***tuberculosis*** library described below in Example 2 and a full · length copy of the M. ***tuberculosis*** homologue was obtained (SEQ ID No. 99).

DETD [0478] The amino acid sequence for antigen (j) was found to be homologous to a known M. ***tuberculosis*** protein translated from a DNA sequence. To the best of the inventors' knowledge, this protein has not been previously shown. . .

DETD Use of Patient Sera to Isolate M. ***tuberculosis*** Antigens

DETD [0481] This example illustrates the isolation of antigens from M. ***tuberculosis*** lysate by screening with serum from M. ***tuberculosis*** -infected individuals.

DETD [0482] Dessicated M. ***tuberculosis*** H37Ra (Difco Laboratories)

was added to a 2% NP40 solution, and alternately homogenized and sonicated three times. The resulting suspension. . . Centriprep 10 (Amicon, Beverley, Mass.) and then screened by Western blot for serological activity using a serum pool from M. ***tuberculosis*** -infected patients which was not immunoreactive with other antigens of the present invention.

DETD [0486] A DNA sequence that encodes the antigen designated as (m) above was obtained by screening a genomic M. ***tuberculosis*** Erdman strain library using labeled degenerate oligonucleotides corresponding to the N-terminal sequence of SEQ ID NO: 137. A clone was. . . NO: 204. Comparison of these sequences with those in the genebank revealed some similarity to sequences previously identified in M. ***tuberculosis*** and M. bovis.

DETD Preparation of DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD [0487] This example illustrates the preparation of DNA sequences encoding M. ***tuberculosis*** antigens by screening a M. ***tuberculosis*** expression library with sera obtained from patients infected with M. ***tuberculosis*** , or with anti-sera raised against soluble M. ***tuberculosis*** antigens.

DETD [0488] A. Preparation of M. ***tuberculosis*** Soluble Antigens Using Rabbit Anti-Sera Raised Against M. ***tuberculosis*** Supernatant

DETD [0489] Genomic DNA was isolated from the M. ***tuberculosis*** strain H37Ra. The DNA was randomly sheared and used to construct an expression library using the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Rabbit anti-sera was generated against secretory proteins of the M. ***tuberculosis*** strains H37Ra, H37Rv and Erdman by immunizing a rabbit with concentrated supernatant of the M. ***tuberculosis*** cultures. Specifically, the rabbit was first immunized subcutaneously with 200 .mu.g of protein antigen in a total volume of 2. . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD [0490] Thirty two clones were purified. Of these, 25 represent sequences that have not been previously identified in human M. ***tuberculosis*** . Recombinant antigens were expressed and purified antigens used in the immunological analysis described in Example 1. Proteins were induced by. . .

DETD . . . ID Nos. 76, 68, 70, 75) show some homology to sequences previously identified in Mycobacterium leprae but not in M. ***tuberculosis*** . TbRA2A was found to be a lipoprotein, with a six residue lipidation sequence being located adjacent to a hydrophobic secretory sequence. TbRA11, TbRA26, TbRA28 and TbDPEP (SEQ ID Nos.: 65, 73, 74, 53) have been previously identified in M. ***tuberculosis*** . No significant homologies were found to TbRA1, TbRA3, TbRA4, TbRA9, TbRA10, TbRA13, TbRA17, TbRa19, TbRA29, TbRA32, TbRA36 and the overlapping. . .

DETD . . . results of PBMC proliferation and interferon-.gamma. assays performed on representative recombinant antigens, and using T-cell preparations from several different M. ***tuberculosis*** -immune patients, are presented in Tables 2 and 3, respectively.

TABLE 2

RESULTS OF PBMC PROLIFERATION TO

REPRESENTATIVE SOLUBLE ANTIGENS

Patient

Antigen 1 2. . .

DETD [0495] These results indicate that these soluble antigens can induce proliferation and/or interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** -immune individual.

DETD [0496] B. Use of Sera From Patients Having Pulmonary or Pleural ***Tuberculosis*** to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD . . . DNA library described above, and an additional H37Rv library, were screened using pools of sera obtained from patients with active ***tuberculosis*** . To prepare the H37Rv library, M. ***tuberculosis*** strain H37Rv genomic DNA was isolated, subjected to partial Sau3A digestion and used to construct an expression library using the. . . and TbH=high reactivity) in both ELISA and immunoblot format. A fourth pool of sera from seven patients with active pulmonary ***tuberculosis*** was also employed. All of the sera lacked increased reactivity with the recombinant 38 kD M. ***tuberculosis*** H37Ra phosphate-binding protein.

DETD . . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD [0499] Thirty two clones were purified. Of these, 31 represented sequences that had not been previously identified in human M. ***tuberculosis*** . Representative sequences of the DNA molecules identified are provided in SEQ ID Nos.: 26-51 and 105. Of these, TbH-8-2 (SEQ.. . . of the open reading frame for the antigen ESAT-6 previously identified in M. bovis (Acc. No. U34848) and in M. ***tuberculosis*** (Sorensen et al., Infec. Immun. 63:1710-1717, 1995).

DETD . . . sequence of Tb38-1F3 is presented in SEQ. ID. NO. 117. A TbH-9 probe identified three clones in the H37Rv library: ***TbH*** - ***9*** - ***FL*** (SEQ. ID NO. 106), which may be the homologue of TbH-9 (R37Ra), TbH-9-1 (SEQ. ID NO. 108), and TbH-9-4 (SEQ.. . .

DETD [0501] Further screening of the M. ***tuberculosis*** genomic DNA library, as described above, resulted in the recovery of ten additional reactive clones, representing seven different genes. One. . . was determined to be identical to the 14Kd alpha crystallin heat shock protein previously shown to be present in M. ***tuberculosis*** , and a third was determined to be identical to the antigen TbH-8 described above. The determined DNA sequences for the. . . contains the reactive open reading frame), although the 3' end of TbH-29 was found to be identical to the M. ***tuberculosis*** cosmid Y227. TbH-32 and TbH-33 were found to be identical to the previously identified M. ***tuberculosis*** insertion element IS6110 and to the M. ***tuberculosis*** cosmid Y50, respectively. No significant homologies to TbH-30 were found.

DETD . . . and uninduced lysates were run in duplicate on SDS-PAGE and transferred to nitrocellulose filters. Filters were reacted with human M. ***tuberculosis*** sera (1:200 dilution) reactive with TbH and a rabbit sera (1:200 or 1:250 dilution) reactive with the N-terminal 4 Kd. . .

DETD [0503] Positive reaction of the recombinant human M. ***tuberculosis*** antigens with both the human M. ***tuberculosis*** sera and anti-lacZ sera indicate that reactivity of

the human M. ***tuberculosis*** sera is directed towards the fusion protein. Antigens reactive with the anti-lacZ sera but not with the human M. ***tuberculosis*** sera may be the result of the human M. ***tuberculosis*** sera recognizing conformational epitopes, or the antigen-antibody binding kinetics may be such that the 2 hour sera exposure in the. . .

DETD [0507] These results indicate that both the inventive M. ***tuberculosis*** antigens and ESAT-6 can induce proliferation and/or interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** -immune individual. To the best of the inventors' knowledge, ESAT-6 has not been previously shown to stimulate human immune responses

DETD . . . help to localize T-cell epitopes within Th38-1 capable of inducing proliferation and interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** immune individual.

TABLE 7

RESULTS OF PBMC PROLIFERATION TO TB38-1 PEPTIDES

Patient

Peptide 1 2 3 4 5 6 7 8 9. . .

DETD [0510] Studies were undertaken to determine whether the antigens TbH-9 and Tb38-1 represent cellular proteins or are secreted into M. ***tuberculosis*** culture media. In the first study, rabbit sera were raised against A) secretory proteins of M. ***tuberculosis*** , B) the known secretory recombinant M. ***tuberculosis*** antigen 85b, C) recombinant Tb38-1 and D) recombinant TbH-9, using protocols substantially the same as that as described in Example 3A. Total M. ***tuberculosis*** lysate, concentrated supernatant of M. ***tuberculosis*** cultures and the recombinant antigens 85b, TbH-9 and Tb38-1 were resolved on denaturing gels, immobilized on nitrocellulose membranes and duplicate. . .

DETD . . . FIGS. 3A-D, respectively, wherein the lane designations are as follows: 1) molecular weight protein standards; 2) 5 .mu.g of M. ***tuberculosis*** lysate; 3) 5 .mu.g secretory proteins; 4) 50 ng recombinant Tb38-1; 5) 50 ng recombinant TbH-9; and 6) 50 ng. . . by an arrow). These results demonstrate that Tb38-1 and TbH-9 are intracellular antigens and are not actively secreted by M. ***tuberculosis*** .

DETD . . . an intracellular antigen was confirmed by determining the reactivity of TbH-9-specific human T cell clones to recombinant TbH-9, secretory M. ***tuberculosis*** proteins and PPD. A TbH-9-specific T cell clone (designated 131TbH-9) was generated from PBMC of a healthy PPD-positive donor. The proliferative response of 131TbH-9 to secretory proteins, recombinant TbH-9 and a control M. ***tuberculosis*** antigen, TbRa11, was determined by measuring uptake of tritiated thymidine, as described in Example 1. As shown in FIG. 4A, the clone 131TbH-9 responds specifically to TbH-9, showing that TbH-9 is not a significant component of M. ***tuberculosis*** secretory proteins. FIG. 4B shows the production of IFN-.gamma. by a second TbH-9-specific T cell clone (designated PPD 800-10) prepared. . . cell clone with secretory proteins, PPD or recombinant TbH-9. These results further confirm that TbH-9 is not secreted by M. ***tuberculosis*** .

DETD [0513] C. Use of Sera From Patients Having Extrapulmonary ***Tuberculosis*** to Identify DNA Sequences Encoding M.

tuberculosis Antigens

DETD [0514] Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). The resulting library was screened using pools of sera obtained from individuals with extrapulmonary ***tuberculosis*** , as described above in Example 3B, with the secondary antibody being goat anti-human IgG +A +M (H+L) conjugated with alkaline. . .

DETD . . . the exception of the 3' ends of XP2 and XP6 which were found to bear some homology to known M. ***tuberculosis*** cosmids. The DNA sequences for XP27 and XP36 are shown in SEQ ID Nos.: 163 and 164, respectively, with the. . .

DETD . . . described herein, recombinant XP1 was found to stimulate cell proliferation and IFN-.gamma. production in T cells isolated from an M. ***tuberculosis*** -immune donors.

DETD [0518] D. Use of a Lysate Positive Serum Pool From Patients Having ***Tuberculosis*** to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD [0519] Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda Screen expression system (Novagen, Madison, Wis.), as described below in Example 6. Pooled serum obtained from M. ***tuberculosis*** -infected patients and that was shown to react with M. ***tuberculosis*** lysate but not with the previously expressed proteins 38kD, Tb38-1, TbRa3, TbH4, DPEP and TbRa11, was used to screen the. . .

DETD . . . SEQ ID NO: 257-260. The remaining seventeen clones were found to show similarities to unknown sequences previously identified in M. ***tuberculosis*** . The determined 5' cDNA sequences for sixteen of these clones (hereinafter referred to as LSER-1, LSER-3, LSER-4, LSER-5, LSER-6, LSER-8,. . .

DETD [0521] E. Preparation of M. ***tuberculosis*** Soluble Antigens Using Rabbit Anti-Sera Raised Against M. ***tuberculosis*** Fractionated Proteins

DETD [0522] M. ***tuberculosis*** lysate was prepared as described above in Example 2. The resulting material was fractionated by HPLC and the fractions screened by Western blot for serological activity with a serum pool from M. ***tuberculosis*** -infected patients which showed little or no immunoreactivity with other antigens of the present invention. Rabbit anti-sera was generated against the most reactive fraction using the method described in Example 3A . The anti-sera was used to screen an M. ***tuberculosis*** Erdman strain genomic DNA expression library prepared as described above. Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones determined.

DETD . . . Of these, one was found to be TbRa35, described above, and one was found to be the previously identified M. ***tuberculosis*** antigen, HSP60. Of the remaining eight clones, seven (hereinafter referred to as RDIF2, RDIF5, RDIF8, RDIF10, RDIF11 and RDIF 12) were found to bear some similarity to previously identified M. ***tuberculosis*** sequences. The determined DNA sequences for RDIF2, RDIF5, RDIF8, RDIF10 and RDIF11 are provided in SEQ ID Nos.: 189-193, respectively,. . .

DETD . . . 8A-B and 9A-B, these antigens were found to stimulate cell

proliferation and IFN-.gamma. production in T cells isolated from M. ***tuberculosis*** -immune donors.

DETD [0525] An M. ***tuberculosis*** polypeptide was isolated from tuberculin purified protein derivative (PPD) as follows.

DETD . . . F. et al., Tuberculin purified protein derivative. Preparation and analyses of a large quantity for standard. The American Review of ***Tuberculosis*** 44:9-25, 1941).

DETD [0527] M. ***tuberculosis*** Rv strain was grown for 6 weeks in synthetic medium in roller bottles at 37.degree. C. Bottles containing the bacterial. . .

DETD [0528] Six fractions were collected, dried, suspended in PBS and tested individually in M. ***tuberculosis*** -infected guinea pigs for induction of delayed type hypersensitivity (DTH) reaction. One fraction was found to induce a strong DTH reaction. . . 80 .mu.l/minute. Eluent was monitored at 215 nm. Eight fractions were collected and tested for induction of DTH in M. ***tuberculosis*** -infected guinea pigs. One fraction was found to induce strong DTH of about 16 mm induration. The other fractions did not. . .

DETD . . . were isolated and found to have the sequences shown in SEQ ID Nos.: 130-133. A subsequent search of the M. ***tuberculosis*** genome database released by the Institute for Genomic Research revealed a match of the DPPD partial amino acid sequence with a sequence present within the M. ***tuberculosis*** cosmid MTY21C12. An open reading frame of 336 bp was identified. The full-length DNA sequence for DPPD is provided in. . .

DETD Use of Sera From ***Tuberculosis*** -Infected Monkeys to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD [0531] Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Serum samples were obtained from a cynomolgous monkey 18, 33, 51 and 56 days following infection with M. ***tuberculosis*** Erdman strain. These samples were pooled and used to screen the M. ***tuberculosis*** genomic DNA expression library using the procedure described above in Example 3C.

DETD . . . MO-35 were found to show a high degree of relatedness and showed some homology to a previously identified unknown M. ***tuberculosis*** sequence and to cosmid MTC1237. MO-2 was found to show some homology to aspartokinase from M. ***tuberculosis*** . Clones MO-3, MO-7 and MO-27 were found to be identical and to show a high degree of relatedness to MO-5. All four of these clones showed some homology to M. ***tuberculosis*** heat shock protein 70. MO-27 was found to show some homology to M. ***tuberculosis*** cosmid MTCY339. MO-4 and MO-34 were found to show some homology to cosmid SCY21B4 and M. smegmatis integration host factor, and were both found to show some homology to a previously identified, unknown M. ***tuberculosis*** sequence. MO-6 was found to show some homology to M. ***tuberculosis*** heat shock protein 65. MO-8, MO-9, MO-10, MO-26 and MO-29 were found to be highly related to each other and to show some homology to M. ***tuberculosis*** dihydrolipamide succinyltransferase. MO-28, MO-31 and MO-32 were found to be identical and to show some homology to a previously identified M. ***tuberculosis*** protein. MO-33 was found to show some homology to a previously identified 14 kDa M. ***tuberculosis*** heat shock protein.

DETD . . . homologies to MO-39. MO-12, MO-13 and MO-19 were found to show some homologies to unknown sequences previously isolated from M. ***tuberculosis*** .

DETD Isolation of DNA Sequences Encoding M. ***tuberculosis*** Antigens by Screening of a Novel Expression Library

DETD [0535] This example illustrates isolation of DNA sequences encoding M. ***tuberculosis*** antigens by screening of a novel expression library with sera from M. ***tuberculosis*** -infected patients that were shown to be unreactive with a panel of the recombinant M. ***tuberculosis*** antigens TbRa11, TbRa3, Tb38-1, TbH4, TbF and 38 kD.

DETD [0536] Genomic DNA from M. ***tuberculosis*** Erdman strain was randomly sheared to an average size of 2 kb, and blunt ended with Klenow polymerase, followed by. . . Wis.) and packaged in vitro using the PhageMaker extract (Novagen). The resulting library was screened with sera from several M. ***tuberculosis*** donors that had been shown to be negative on a panel of previously identified M. ***tuberculosis*** antigens as described above in Example 3B.

DETD . . . 330-332, 334, 336, 338, 340 and 342 were found to show some homology to unknown sequences previously identified in M. ***tuberculosis*** .

DETD Isolation of Soluble M. ***tuberculosis*** Antigens Using Mass Spectrometry

DETD [0538] This example illustrates the use of mass spectrometry to identify soluble M. ***tuberculosis*** antigens.

DETD [0539] In a first approach, M. ***tuberculosis*** culture filtrate was screened by Western analysis using serum from a ***tuberculosis*** -infected individual. The reactive bands were excised from a silver stained gel and the amino acid sequences determined by mass spectrometry.. . . of this sequence with those in the gene bank revealed homology to the 85b precursor antigen previously identified in M. ***tuberculosis*** .

DETD [0540] In a second approach, the high molecular weight region of M. ***tuberculosis*** culture supernatant was studied. This area may contain immunodominant antigens which may be useful in the diagnosis of M. ***tuberculosis*** infection. Two known monoclonal antibodies, IT42 and IT57 (available from the Center for Disease Control, Atlanta, Ga.), show reactivity by. . . the antigens remains unknown. In addition, unknown high-molecular weight proteins have been described as containing a surrogate marker for M. ***tuberculosis*** infection in HIV-positive individuals (Jnl. Infect. Dis., 176:133-143, 1997). To determine the identity of these antigens, two-dimensional gel electrophoresis and. . .

DETD . . . phosphoenolpyruvate kinase. The two sequences isolated from spot 2 were determined to be from two DNAks, previously identified in M. ***tuberculosis*** as heat shock proteins. Spot 4 was determined to be the previously identified M. ***tuberculosis*** protein Kat G. To the best of the inventors' knowledge, neither PcK-1 nor the two DNAks have previously been shown to have utility in the diagnosis of M. ***tuberculosis*** infection.

DETD Use of Representative Antigens for Diagnosis of ***Tuberculosis***

DETD [0542] This example illustrates the effectiveness of several representative polypeptides in skin tests for the diagnosis of M. ***tuberculosis*** infection.

DETD . . . 20 individuals tested, 2 were PPD negative and 18 were PPD

positive. Of the PPD positive individuals, 3 had active ***tuberculosis*** , 3 had been previously infected with ***tuberculosis*** and 9 were healthy. In a second study, 13 PPD positive individuals were tested with 0.1 .mu.g TbRa11 in either. . .

DETD Preparation and Characterization of M. ***tuberculosis*** Fusion Proteins

DETD [0555] The reactivity of the fusion protein TbF-2 with sera from M. ***tuberculosis*** -infected patients was examined by ELISA using the protocol described above. The results of these studies (Table 11) demonstrate that all. . .

DETD [0557] Genomic M. ***tuberculosis*** DNA was used to PCR full-length TbH4 (FL TbH4) with the primers PDM-157 and PDM-160 (SEQ ID NO: 348 and. . .

DETD SEQUENCE CHARACTERISTICS:
LENGTH: 53 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 146
GGATCCATAT GGGCCATCAT CATCATCATC ACGTGATCGA CATCATCGGG ACC 53

DETD SEQUENCE CHARACTERISTICS:
LENGTH: 42 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR Primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 147
CCTGAATTCA GGCCTCGGTT GCGCCGGCCT CATCTTGAAC GA 42

DETD SEQUENCE CHARACTERISTICS:
LENGTH: 31 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR Primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 148
GGATCCTGCA GGCTCGAAAC CACCGAGCGG T 31

DETD SEQUENCE CHARACTERISTICS:
LENGTH: 31 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***

SEQUENCE: 149
CTCTGAATTC AGCGCTGGAA ATCGTCGCGA T 31
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 33 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 150
GGATCCAGCG CTGAGATGAA GACCGATGCC GCT 33
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 33 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 151
GAGAGAATTC TCAGAAGCCC ATTTGCGAGG ACA 33
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 1993 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: DNA (genomic)
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
FEATURE:
NAME/KEY: CDS
LOCATION: 152..1273
SEQUENCE: 152
TGTTCTTCGA CGGCAGGCTG GTGGAGGAAG GGCCCACCGA ACAGCTGTTC TCCTCGCCGA 60
AGCATGCGGA AACCGCCCGA TACGTCGCCG GACTGTCGGG GGACGTCAAG GACGCCAAGC 120
GCGGAAATTG AAGAGCACAG AAAGGTATGG. . .
CLM What is claimed is:

1. A polypeptide comprising an immunogenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen has an. . .
2. A polypeptide comprising an immunogenic portion of an M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen has an. . .
3. A polypeptide comprising an immunogenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an. . .
4. A polypeptide comprising an immunogenic portion of a M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said

antigen comprises an. . .
23. A fusion protein comprising one or more polypeptides according to any one of claims 1-4 and the M. ***tuberculosis*** antigen 38 kD (SEQ ID NO: 155).

29. A method for detecting ***tuberculosis*** in a patient, comprising: (a) contacting dermal cells of a patient with one or more polypeptides according to any one of claims 14; and (b) detecting an immune response on the patient's skin and therefrom detecting ***tuberculosis*** in the patient.

30. A method for detecting ***tuberculosis*** in a patient, comprising: (a) contacting dermal cells of a patient with a polypeptide having an N-terminal sequence selected from. . . in SEQ ID NO: 134 and 135; and (b) detecting an immune response on the patient's skin and therefrom detecting ***tuberculosis*** in the patient.

31. A method for detecting ***tuberculosis*** in a patient, comprising: (a) contacting dermal cells of a patient with one or more polypeptides encoded by a DNA. . . 330-332, 334, 336, 338, 340 and 342-347; and (b) detecting an immune response on the patient's skin and therefrom detecting ***tuberculosis*** in the patient.

L15 ANSWER 2 OF 7 USPATFULL on STN
AN 2003:195215 USPATFULL
TI Compounds and methods for diagnosis of ***tuberculosis***
IN Reed, Steven G., Bellevue, WA, UNITED STATES
Skeiky, Yasir A.W., Seattle, WA, UNITED STATES
Dillon, Davin C., Redmond, WA, UNITED STATES
Campos-Neto, Antonio, Bainbridge Island, WA, UNITED STATES
Houghton, Raymond, Bothell, WA, UNITED STATES
Vedvick, Thomas S., Federal Way, WA, UNITED STATES
Twardzik, Daniel R., Bainbridge Island, WA, UNITED STATES
Lodes, Michael J., Seattle, WA, UNITED STATES
Hendrickson, Ronald C., Seattle, WA, UNITED STATES
PA Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)
PI US 2003135026 A1 20030717
AI US 2002-193002 A1 20020710 (10)
RLI Continuation of Ser. No. US 1998-72596, filed on 5 May 1998, GRANTED, Pat. No. US 6458366 Continuation-in-part of Ser. No. US 1998-24753, filed on 18 Feb 1998, ABANDONED Continuation-in-part of Ser. No. US 1997-942341, filed on 1 Oct 1997, ABANDONED Continuation-in-part of Ser. No. US 1997-818111, filed on 13 Mar 1997, GRANTED, Pat. No. US 6338852 Continuation-in-part of Ser. No. US 1996-729622, filed on 11 Oct 1996, ABANDONED A 371 of International Ser. No. WO 1996-US14675, filed on 30 Aug 1996, PENDING A 371 of International Ser. No. US 1996-680574, filed on 12 Jul 1996, ABANDONED Continuation-in-part of Ser. No. US 1996-658800, filed on 5 Jun 1996, ABANDONED Continuation-in-part of Ser. No. US 1996-620280, filed on 22 Mar 1996, ABANDONED Continuation-in-part of Ser. No. US 1995-532136, filed on 22 Sep 1995, ABANDONED Continuation of Ser. No. US 1995-523435, filed on 1 Sep 1995, ABANDONED
DT Utility
FS APPLICATION
LREP TOWNSEND AND TOWNSEND AND CREW, LLP, TWO EMBARCADERO CENTER, EIGHTH

FLOOR, SAN FRANCISCO, CA, 94111-3834
CLMN Number of Claims: 54
ECL Exemplary Claim: 1
DRWN 19 Drawing Page(s)
LN.CNT 9455
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Compounds and methods for diagnosing ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of one or more M. ***tuberculosis*** proteins, and DNA sequences encoding such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of M. ***tuberculosis*** infection in patients and biological samples. Antibodies directed against such polypeptides are also provided.
TI Compounds and methods for diagnosis of ***tuberculosis***
AB Compounds and methods for diagnosing ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of one or more M. ***tuberculosis*** proteins, and DNA sequences encoding such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of M. ***tuberculosis*** infection in patients and biological samples. Antibodies directed against such polypeptides are also provided.
SUMM [0002] The present invention relates generally to the detection of Mycobacterium ***tuberculosis*** infection. The invention is more particularly related to polypeptides comprising a Mycobacterium ***tuberculosis*** antigen, or a portion or other variant thereof, and the use of such polypeptides for the serodiagnosis of Mycobacterium ***tuberculosis*** infection.
SUMM [0003] ***Tuberculosis*** is a chronic, infectious disease, that is generally caused by infection with Mycobacterium ***tuberculosis*** . It is a major disease in developing countries, as well as an increasing problem in developed areas of the world,. . .
SUMM [0004] Although ***tuberculosis*** can generally be controlled using extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease.. . .
SUMM [0005] Inhibiting the spread of ***tuberculosis*** will require effective vaccination and accurate, early diagnosis of the disease. Currently, vaccination with live bacteria is the most efficient. . .
SUMM [0006] While macrophages have been shown to act as the principal effectors of M. ***tuberculosis*** immunity, T cells are the predominant inducers of such immunity. The essential role of T cells in protection against M. ***tuberculosis*** infection is illustrated by the frequent occurrence of M. ***tuberculosis*** in AIDS patients, due to the depletion of CD4 T cells associated with human immunodeficiency virus (HIV) infection. Mycobacterium-reactive CD4. . . that 1,25-dihydroxy-vitamin D3, either alone or in combination with IFN-.gamma. or tumor necrosis factor-alpha, activates human macrophages to inhibit M. ***tuberculosis*** infection. Furthermore, it is known that IFN-.gamma. stimulates human macrophages to make 1,25dihydroxy-vitamin D3. Similarly, IL-12 has been shown to play a role in stimulating resistance to M. ***tuberculosis*** infection. For a review of the immunology of M. ***tuberculosis*** infection see Chan and Kaufmann, in ***Tuberculosis*** : Pathogenesis, Protection and Control, Bloom (ed.), ASM Press, Washington, D.C., 1994.

SUMM [0007] Accordingly, there is a need in the art for improved diagnostic methods for detecting ***tuberculosis*** . The present invention fulfills this need and further provides other related advantages.

SUMM [0008] Briefly stated, the present invention provides compositions and methods for diagnosing ***tuberculosis*** . In one aspect, polypeptides are provided comprising an antigenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. In one embodiment of. . .

SUMM [0010] In a related aspect, polypeptides are provided comprising an immunogenic portion of an M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, the antigen having one. . .

SUMM [0012] In another embodiment, the soluble M. ***tuberculosis*** antigen comprises an amino acid sequence encoded by a DNA sequence selected from the group consisting of the sequences recited. . .

SUMM [0013] In a related aspect, the polypeptides comprise an antioenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, wherein the antigen comprises. . .

SUMM . . . provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, an inventive polypeptide and a known M. ***tuberculosis*** antigen.

SUMM [0016] In further aspects of the subject invention, methods and diagnostic kits are provided for detecting ***tuberculosis*** in a patient The methods comprise: (a) contacting a biological sample with at least one of the above polypeptides; and (b) detecting in the sample the presence of antibodies that bind to the polypeptide or polypeptides, thereby detecting M. ***tuberculosis*** infection in the biological sample. Suitable biological samples include whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine. The. . .

SUMM [0017] The present invention also provides methods for detecting M. ***tuberculosis*** infection comprising: (a) obtaining a biological sample from a patient; (b) contacting the sample with at least one oligonucleotide primer. . .

SUMM [0018] In a further aspect, the present invention provides a method for detecting M. ***tuberculosis*** infection in a patient comprising: (a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide. . .

SUMM . . . monoclonal, that bind to the polypeptides described above, as well as methods for their use in the detection of M. ***tuberculosis*** infection.

DRWD . . . illustrate the stimulation of proliferation and interferon-.gamma. .gamma. production in T cells derived from a first and a second M. ***tuberculosis*** --immune donor, respectively, by the 14 Kd, 20 Kd and 26 Kd antigens described in Example 1.

DRWD [0022] FIGS. 2A-D illustrate the reactivity of antisera raised against secretory M. ***tuberculosis*** proteins, the known M. ***tuberculosis*** antigen 85b and the inventive antigens Tb38-1 and TbH-9, respectively, with M. ***tuberculosis*** lysate (lane 2), M. ***tuberculosis*** secretory proteins (lane 3), recombinant Tb38-1 (lane 4), recombinant TbH-9 (lane 5) and recombinant 85b (lane 5).

DRWD [0023] FIG. 3A illustrates the stimulation of proliferation in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, recombinant TbH-9 and a control antigen, TbRa11.

DRWD [0024] FIG. 3B illustrates the stimulation of interferon-.gamma. production in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, PPD and recombinant TbH-9.

DRWD [0025] FIG. 4 illustrates the reactivity of two representative polypeptides with sera from M. ***tuberculosis*** --infected and uninfected individuals, as compared to the reactivity of bacterial lysate.

DRWD [0026] FIG. 5 shows the reactivity of four representative polypeptides with sera from M. ***tuberculosis*** -infected and uninfected individuals, as compared to the reactivity of the 38 kD antigen.

DRWD [0027] FIG. 6 shows the reactivity of recombinant 38 kD and ThRa11 antigens with sera from M. ***tuberculosis*** patients, PPD positive donors and normal donors.

DRWD [0029] FIG. 8 shows the reactivity of the antigen of SEQ ID NO: 60 with sera from M. ***tuberculosis*** patients and normal donors.

DRWD [0030] FIG. 9 illustrates the reactivity of the recombinant antigen TbH-29 (SEQ ID NO: 137) with sera from M. ***tuberculosis*** patients, PPD positive donors and normal donors as determined by indirect ELISA.

DRWD [0031] FIG. 10 illustrates the reactivity of the recombinant antigen TbH-33 (SEQ ID NO: 140) with sera from M. ***tuberculosis*** patients and from normal donors, and with a pool of sera from M. ***tuberculosis*** patients, as determined both by direct and indirect ELISA FIG. 11 illustrates the reactivity of increasing concentrations of the recombinant antigen TbH-33 (SEQ ID NO: 140) with sera from M. ***tuberculosis*** patients and from normal donors as determined by ELISA.

DRWD . . . FIGS. 12A-E illustrate the reactivity of the recombinant antigens MO-1, MO-2, MO-4, MO-28 and MO-29, respectively, with sera from M. ***tuberculosis*** patients and from normal donors as determined by ELISA.

DETD [0173] SEQ ID NO: 149 is the DNA sequence of the M. ***tuberculosis*** antigen 38 kD.

DETD [0174] SEQ ID NO: 150 is the amino acid sequence of the M. ***tuberculosis*** antigen 38 kD.

DETD [0355] SEQ ID NO: 338 is the determined amino acid sequence for a M. ***tuberculosis*** 85 b precursor homolog

DETD [0368] As noted above, the present invention is generally directed to no compositions and methods for diagnosing ***tuberculosis*** . The compositions of the subject invention include polypeptides that comprise at least one antigenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. Polypeptides within the scope of the present invention include, but are not limited to, soluble M. ***tuberculosis*** antigens. A "soluble M. ***tuberculosis*** antigen" is a protein of M. ***tuberculosis*** origin that is present in M. ***tuberculosis*** culture filtrate. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e.,. . . entirely of the antigenic portion, or may contain additional sequences. The additional sequences may be derived from the native M. ***tuberculosis*** antigen or may be heterologous, and such sequences may (but need not) be antigenic.

DETD . . . may or may not be soluble) is a portion that is capable of reacting with sera obtained from an M. ***tuberculosis*** -infected individual (i.e., generates an absorbance reading with sera from

infected individuals that is at least three standard deviations above the absorbance obtained with sera from uninfected individuals, in a representative ELISA assay described herein). An "M. ***tuberculosis*** -infected individual" is a human who has been infected with M. ***tuberculosis*** (e.g. has an intradermal skin test response to PPD that is at least 0.5 cm in diameter). Infected individuals may display symptoms of ***tuberculosis*** or may be free of disease symptoms. Polypeptides comprising at least an antigenic portion of one or more M. ***tuberculosis*** antigens as described herein may generally be used, alone or in combination, to detect ***tuberculosis*** in a patient.

DETD . . . be identified by evaluating a modified polypeptide for the ability to generate antibodies that detect the presence or absence of ***tuberculosis*** . Such modified sequences may be prepared and tested using, for example, the representative procedures described herein.

DETD . . . polypeptide" is a polypeptide comprising at least one of the above antigenic portions and one or more additional antigenic M. ***tuberculosis*** sequences, which are joined via a peptide linkage into a single amino acid chain. The sequences may be joined directly. . .

DETD [0375] In general, M. ***tuberculosis*** antigens, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, soluble antigens may be isolated from M. ***tuberculosis*** culture filtrate by procedures known to those of ordinary skill in the art, including anion-exchange and reverse phase chromatography. Purified. . . may then be evaluated for a desired property, such as the ability to react with sera obtained from an M. ***tuberculosis*** -infected individual. Such screens may be performed using the representative methods described herein. Antigens may then be partially sequenced using, for. . .

DETD . . . and 15 expressed in an appropriate host. DNA molecules encoding soluble antigens may be isolated by screening an appropriate M. ***tuberculosis*** expression library with anti-sera (e.g., rabbit) raised specifically against soluble M. ***tuberculosis*** antigens. DNA sequences encoding antigens that may or may not be soluble may be identified by screening an appropriate M. ***tuberculosis*** genomic or cDNA expression library with sera obtained from patients infected with M. ***tuberculosis*** . Such screens may generally be performed using techniques well known in the art, such as those described in Sambrook et. . .

DETD [0377] DNA sequences encoding soluble antigens may also be obtained by screening an appropriate M. ***tuberculosis*** cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of. . .

DETD . . . antigens described herein are "antigenic." More specifically, the antigens have the ability to react with sera obtained from an M. ***tuberculosis*** -infected individual. Reactivity may be evaluated using, for example, the representative ELISA assays described herein, where an absorbance reading with sera. . .

DETD [0379] Antigenic portions of M. ***tuberculosis*** antigens may be prepared and identified using well known techniques, such as those summarized in Paul, Fundamental Immunology, 3d ed.,. . . is substantially similar to that generated by the full length antigen. In other words, an antigenic portion of a M. ***tuberculosis*** antigen generates at least about 20%, and preferably about 100%, of the signal

induced by the full length antigen in. . .

DETD [0380] Portions and other variants of M. ***tuberculosis*** antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally. . .

DETD [0384] In certain specific embodiments, the subject invention discloses polypeptides comprising at least an antigenic portion of a soluble M. ***tuberculosis*** antigen (or a variant of such an antigen), where the antigen has one of the following N-terminal sequences:

(a) Asp-Pro-Val-Asp-Ala-Val-Ile-Asn-Thr-Thr-Cys-Asn-Tyr-Gly-. . .

DETD [0386] In a further specific embodiment, the subject invention discloses polypeptides comprising at least an immunogenic portion of an M. ***tuberculosis*** antigen having one of the following N-terminal sequences, or a variant thereof that differs only in conservative substitutions and/or modifications:. . .

DETD [0388] In other specific embodiments, the subject invention discloses polypeptides comprising at least an antigenic portion of a soluble M. ***tuberculosis*** antigen (or a variant of such an antigen) that comprises one or more of the amino acid sequences encoded by. . .

DETD [0389] In further specific embodiments, the subject invention discloses polypeptides comprising at least an antigenic portion of a M. ***tuberculosis*** antigen (or a variant of such an antigen), which may or may not be soluble, that comprises one or more. . .

DETD . . . comprising a first and a second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known M. ***tuberculosis*** antigen, such as the 38 kD antigen described in Andersen and Hansen, Infect. Immun. 57:2481-2488, 1989, (Genbank Accession No. M30046). . .

DETD [0393] In another aspect, the present invention provides methods for using the polypeptides described above to diagnose ***tuberculosis*** . In this aspect, methods are provided for detecting M. ***tuberculosis*** infection in a biological sample, using one or more of the above polypeptides, alone or in combination. In embodiments in. . . a predetermined cut-off value. The presence of such antibodies indicates previous sensitization to mycobacterial antigens which may be indicative of ***tuberculosis*** .

DETD . . . using each polypeptide individually to evaluate serum samples obtained from a series of patients known to be infected with M. ***tuberculosis*** . After determining which samples test positive (as described below) with each polypeptide, combinations of two or more polypeptides may be. . . infection in most, or all, of the samples tested. Such polypeptides are complementary. For example, approximately 25-30% of sera from ***tuberculosis*** -infected individuals are negative for antibodies to any single protein, such as the 38 kD antigen mentioned above. Complementary polypeptides may,. . .

DETD . . . (i.e., incubation time) is that period of time that is sufficient to detect the presence of antibody within a M. ***tuberculosis*** -infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that. . .

DETD [0403] To determine the presence or absence of anti M. ***tuberculosis*** antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally. . . general, a sample generating a signal that is three standard

deviations above the predetermined cut-off value is considered positive for ***tuberculosis*** . In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of. . . a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for ***tuberculosis*** .

DETD . . . reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of anti-M. ***tuberculosis*** antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line,. . .

DETD [0409] Antibodies may be used in diagnostic tests to detect the presence of M. ***tuberculosis*** antigens using assays similar to those detailed above and other techniques well known to those of skill in the art, thereby providing a method for detecting M. ***tuberculosis*** infection in a patient.

DETD . . . example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify M. ***tuberculosis*** -specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a DNA molecule. . .

DETD . . . art (see, for example, Mullis et al. Ibid; Ehrlich, Ibid). Primers or probes may thus be used to detect M. ***tuberculosis*** -specific sequences in biological samples. DNA probes or primers comprising oligonucleotide sequences described above may be used alone, in combination with. . .

DETD Purification and Characterization of Polypeptides From M. ***tuberculosis*** Culture Filtrate

DETD [0413] This example illustrates the preparation of M. ***tuberculosis*** soluble polypeptides from culture filtrate. Unless otherwise noted, all percentages in the following example are weight per volume.

DETD [0414] M. ***tuberculosis*** (either H37Ra, ATCC No. 25177, or H37Rv, ATCC No. 25618) was cultured in sterile GAS media at 37.degree. C. for. . .

DETD [0426] Additional soluble antigens were isolated from M. ***tuberculosis*** culture filtrate as follows. M. ***tuberculosis*** culture filtrate was prepared as described above. Following dialysis against Bis-Tris propane buffer, at pH 5.5, fractionation was performed using. . .

DETD . . . DNA sequences that encode the antigens designated as (a), (c), (d) and (g) above were obtained by screening a M. ***tuberculosis*** genomic library using .sup.32P end labeled degenerate oligonucleotides corresponding to the N-terminal sequence and containing M. ***tuberculosis*** codon bias. The screen performed using a probe corresponding to antigen (a) above identified a clone having the sequence provided. . .

DETD . . . was amplified from genomic DNA using the sequence obtained from GENBANK. This sequence was then used to screen an M. ***tuberculosis*** library and a fill length copy of the M. ***tuberculosis*** homologue was obtained (SEQ ID NO: 94).

DETD [0434] The amino acid sequence for antigen (j) was found to be homologous to a known M. ***tuberculosis*** protein translated from a DNA sequence. To the best of the inventors' knowledge, this protein has not been previously shown. . .

DETD Use of Patient Sera to Isolate M. ***tuberculosis*** Antigens

DETD [0437] This example illustrates the isolation of antigens from M. ***tuberculosis*** lysate by screening with serum from M. ***tuberculosis*** -infected individuals.

DETD [0438] Dessicated M. ***tuberculosis*** H37Ra (Difco Laboratories) was added to a 2% NP40 solution, and alternately homogenized and sonicated three times. The resulting suspension. . . a Centriprep 10 (Amicon, Beverley, Mass.) and screened by Western blot for serological activity using a serum pool from M. ***tuberculosis*** -infected patients which was not immunoreactive with other antigens of the present invention.

DETD [0441] A DNA sequence that encodes the antigen designated as (m) above was obtained by screening a genomic M. ***tuberculosis*** Erdman strain library using labeled degenerate oligonucleotides corresponding to the N-terminal sequence of SEQ ID NO: 137. A clone was. . . NO: 199. Comparison of these sequences with those in the genebank revealed some similarity to sequences previously identified in M. ***tuberculosis*** and M. bovis.

DETD Preparation of DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD [0442] This example illustrates the preparation of DNA sequences encoding M. ***tuberculosis*** antigens by screening a M. ***tuberculosis*** expression library with sera obtained from patients infected with M. ***tuberculosis*** , or with anti-sera raised against M. ***tuberculosis*** antigens.

DETD [0443] A. Preparation of M. ***tuberculosis*** Soluble Antigens Using Rabbit Anti-Sera Raised Against M. ***tuberculosis*** Supernatant

DETD [0444] Genomic DNA was isolated from the M. ***tuberculosis*** strain H37Ra. The DNA was randomly sheared and used to construct an expression library using the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Rabbit anti-sera was generated against secretory proteins of the M. ***tuberculosis*** strains H37Ra, H37Rv and Erdman by immunizing a rabbit with concentrated supernatant of the M. ***tuberculosis*** cultures. Specifically, the rabbit was first immunized subcutaneously with 200 .mu.g of protein antigen in a total volume of 2. . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD [0445] Thirty two clones were purified. Of these, 25 represent sequences that have not been previously identified in M. ***tuberculosis*** . Proteins were induced by IPTG and purified by gel elution, as described in Skeiky et al., J. Exp. Med. 181:1527-1537,. . .

DETD . . . ID NOS: 77, 69, 71, 76) show some homology to sequences previously identified in Mycobacterium leprae but not in M. ***tuberculosis*** . TbRA2A was found to be a lipoprotein, with a six residue lipidation sequence being located adjacent to a hydrophobic secretory sequence. TbRA11, TbRA26, TbRA28 and TbDPEP (SEQ ID NOS: 66, 74, 75, 53) have been previously identified in M. ***tuberculosis*** . No significant homologies were found to TbRA1, TbRA3, TbRA4, TbRA9, TbRA10, TbRA13, TbRA17, TbRA19, TbRA29, TbRA32, TbRA36 and the overlapping. . .

DETD B. Use of Sera From Patients Having Pulmonary or Pleural ***Tuberculosis*** to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD . . . DNA library described above, and an additional H37Rv library,

were screened using pools of sera obtained from patients with active ***tuberculosis*** . To prepare the H37Rv library, M. ***tuberculosis*** strain H37Rv genomic DNA was isolated, subjected to partial Sau3A digestion and used to construct an expression library using the. . . and TbH=high reactivity) in both ELISA and immunoblot format. A fourth pool of sera from seven patients with active pulmonary ***tuberculosis*** was also employed. All of the sera lacked increased reactivity with the recombinant 38 kDt M. ***tuberculosis*** H37Ra phosphate-binding protein.

DETD . . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD [0449] Thirty two clones were purified. Of these, 31 represented sequences that had not been previously identified in human M. ***tuberculosis*** . Representative sequences of the DNA molecules identified are provided in SEQ ID NOS: 26-51 and 100. Of these, TbH-8-2 (SEQ. . . of the open reading frame for the antigen ESAT-6 previously identified in M. bovis (Acc. No. U34848) and in M. ***tuberculosis*** (Sorensen et al., Infec. Immun. 63:1710-1717. 1995).

DETD . . . sequence of Tb38-1F3 is presented in SEQ. ID. NO. 112. A TbH-9 probe identified three clones in the H37Rv library: ***TbH*** - ***9*** - ***FL*** (SEQ. ID NO. 101), which may be the homologue of TbH-9 (R37Ra), TbH-9-1 (SEQ. ID NO. 103), and TbH-8-2 (SEQ.. . .

DETD [0453] Further screening of the M. ***tuberculosis*** genomic DNA library, as described above, resulted in the recovery of ten additional reactive clones, representing seven different genes. One. . . determined to be identical to the 14 Kd alpha crystallin heat shock protein previously shown to be present in M. ***tuberculosis*** , and a third was determined to be identical to the antigen TbH-8 described above. The determined DNA sequences for the. . . contains the reactive open reading frame), although the 3' end of TbH-29 was found to be identical to the M. ***tuberculosis*** cosmid Y227. TbH-32 and TbH-33 were found to be identical to the previously identified M. ***tuberculosis*** insertion element IS6110 and to the M. ***tuberculosis*** cosmid Y50, respectively. No significant homologies to TbH-30 were found.

DETD . . . and uninduced lysates were run in duplicate on SDS-PAGE and transferred to nitrocellulose filters. Filters were reacted with human M. ***tuberculosis*** sera (1:200 dilution) reactive with TbH and a rabbit sera (1:200 or 1:250 dilution) reactive with the N-terminal 4 Kd. . .

DETD [0455] Positive reaction of the recombinant human M. ***tuberculosis*** antigens with both the human M. ***tuberculosis*** sera and anti-lacZ sera indicate that reactivity of the human M. ***tuberculosis*** sera is directed towards the fusion protein. Antigens reactive with the anti-lacZ sera but not with the human M. ***tuberculosis*** sera may be the result of the human M. ***tuberculosis*** sera recognizing conformational epitopes, or the antigen-antibody binding kinetics may be such that the 2 hour sera exposure in the. . .

DETD [0456] Studies were undertaken to determine whether the antigens TbH-9 and Tb38-1 represent cellular proteins or are secreted into M. ***tuberculosis*** culture media In the first study, rabbit sera were raised against A) secretory proteins of M. ***tuberculosis*** , B) the known secretory recombinant M. ***tuberculosis*** antigen 85b,

C) recombinant Tb38-1 and D) recombinant TbH-9, using protocols substantially as described in Example 3A. Total M. ***tuberculosis*** lysate, concentrated supernatant of M. ***tuberculosis*** cultures and the recombinant antigens 85b, TbH-9 and Tb38-1 were resolved on denaturing gels, immobilized on nitrocellulose membranes and duplicate. . .

DETD . . . FIGS. 2A-D, respectively, wherein the lane designations are as follows: 1) molecular weight protein standards; 2) 5 .mu.g of M. ***tuberculosis*** lysate; 3) 5 .mu.g secretory proteins; 4) 50 ng recombinant Tb38-1; 5) 50 ng recombinant TbH-9; and 6) 50 ng. . . by an arrow). These results demonstrate that Tb38-1 and TbH-9 are intracellular antigens and are not actively secreted by M. ***tuberculosis*** .

DETD . . . an intracellular antigen was confirmed by determining the reactivity of TbH-9-specific human T cell clones to recombinant TbH-9, secretory M. ***tuberculosis*** proteins and PPD. A TbH-9-specific T cell clone (designated 131TbH-9) was generated from PBMC of a healthy PPD-positive donor. The proliferative response of 131TbH-9 to secretory proteins, recombinant TbH-9 and a control M. ***tuberculosis*** antigen, TbRa11, was determined by measuring uptake of tritiated thymidine, as described in Example 1. As shown in FIG. 3A, the clone 131TbH-9 responds specifically to TbH-9, showing that TbH-9 is not a significant component of M. ***tuberculosis*** secretory proteins. FIG. 3B shows the production of IFN-.gamma. by a second TbH-9-specific T cell clone (designated PPD 800-10) prepared. . . cell clone with secretory proteins, PPD or recombinant TbH-9. These results further confirm that TbH-9 is not secreted by M. ***tuberculosis*** .

DETD C. Use of Sera From Patients Having Extrapulmonary ***Tuberculosis*** to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD [0459] Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). The resulting library was screened using pools of sera obtained from individuals with extrapulmonary ***tuberculosis*** , as described above in Example 3B, with the secondary antibody being goat anti-human IgG+A+M (H+L) conjugated with alkaline phosphatase.

DETD . . . the exception of the 3' ends of XP2 and XP6 which were found to bear some homology to known M. ***tuberculosis*** cosmids. The DNA sequences for XP27 and XP36 are shown in SEQ ID NOS: 158 and 159, respectively, with the. . .

DETD . . . for purification. Recombinant XP1 was found to stimulate cell proliferation and IFN-.gamma. production in T cells isolated from an M. ***tuberculosis*** -immune donors.

DETD D. Use of a Lysate Positive Serum Pool From Patients Having ***Tuberculosis*** to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigenes

DETD [0463] Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda Screen expression system (Novagen, Madison, Wis.), as described below in Example 6. Pooled serum obtained from M. ***tuberculosis*** --infected patients and that was shown to react with M. ***tuberculosis*** lysate but not with the previously expressed proteins 38 kD, Tb38-1, TbRa3. TbH4, DPEP and TbRa11, was used to screen. . .

DETD . . . SEQ ID NO: 252-255. The remaining seventeen clones were found

to show similarities to unknown sequences previously identified in M. ***tuberculosis*** . The determined 5' cDNA sequences for sixteen of these clones (hereinafter referred to as LSER-1, LSER-3, LSER-4, LSER-5, LSER-6, LSER-8,. . .

DETD E. Preparation of M. ***tuberculosis*** Soluble Antigens Using Rabbit Anti-Sera Raised Against M. ***tuberculosis*** Fractionated Proteins

DETD [0465] M. ***tuberculosis*** lysate was prepared as described above in Example 2. The resulting material was fractionated by HPLC and the fractions screened by Western blot for serological activity with a serum pool from M. ***tuberculosis*** --infected patients which showed little or no immunoreactivity with other antigens of the present invention. Rabbit anti-sera was generated against the most reactive fraction using the method described in Example 3A. The anti-sera was used to screen an M. ***tuberculosis*** Erdman strain genomic DNA expression library prepared as described above. Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones determined.

DETD . . . Of these, one was found to be TbRa35, described above, and one was found to be the previously identified M. ***tuberculosis*** antigen, HSP60. Of the remaining eight clones, six (hereinafter referred to as RDIF2, RDIF5, RDIF8, RDLF10, RDIF11 and RDIF12) were found to bear some similarity to previously identified M. ***tuberculosis*** sequences. The determined DNA sequences for RDIF2, RDIF5, RDIF8, RDIF10 and RDIF11 are provided in SEQ ID NOS: 184-188, respectively,. . .

DETD . . . as described above. These antigens were found to stimulate cell proliferation and IFN-.gamma. production in T cells isolated from M. ***tuberculosis*** -immune donors.

DETD [0468] An M. ***tuberculosis*** polypeptide was isolated from tuberculin purified protein derivative (PPD) as follows.

DETD . . . F. et al., Tuberculin purified protein derivative. Preparation and analyses of a large quantity for standard. The American Review of ***Tuberculosis*** 44:9-25, 1941). M. ***tuberculosis*** Rv strain was grown for 6 weeks in synthetic medium in roller bottles at 37.degree. C. Bottles containing the bacterial. . .

DETD [0470] Six fractions were collected, dried, suspended in PBS and tested individually in M. ***tuberculosis*** -infected guinea pigs for induction of delayed type hypersensitivity (DTH) reaction. One fraction was found to induce a strong DTH reaction. . . 80 .mu.l/minute. Eluent was monitored at 215 rm. Eight fractions were collected, and tested for induction of DTH in M. ***tuberculosis*** -infected guinea pigs. One fraction was found to induce strong DTH of about 16 mm induration. The other fractions did not. . .

DETD . . . were isolated and found to have the sequences shown in SEQ ID NOS: 125-128. A subsequent search of the M. ***tuberculosis*** genome database released by the Institute for Genomic Research revealed a match of the DPPD partial amino acid sequence with a sequence present within the M. ***tuberculosis*** cosrid MTY21C12. An open reading frame of 336 bp was identified. The full-length DNA sequence for DPPD is provided in. . .

DETD Use of Sera From ***Tuberculosis*** -Infected Monkeys to Identify DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD [0472] Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library

employing the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Serum samples were obtained from a cynomolgous monkey 18, 33, 51 and 56 days following infection with M. ***tuberculosis*** Erdman strain. These samples were pooled and used to screen the M. ***tuberculosis*** genomic DNA expression library using the procedure described above in Example 3C.

DETD . . . MO-35 were found to show a high degree of relatedness and showed some homology to a previously identified unknown M. ***tuberculosis*** sequence and to cosmid MTC1237. MO-2 was found to show some homology to aspartokinase from M. ***tuberculosis*** . Clones MO-3, MO-7 and MO-27 were found to be identical and to show a high degree of relatedness to MO-5. All four of these clones showed some homology to M. ***tuberculosis*** heat shock protein 70. MO-27 was found to show some homology to M. ***tuberculosis*** cosmid MTCY339. MO-4 and MO-34 were found to show some homology to cosmid SCY21B4 and M. smegmatis integration host factor, and were both found to show some homology to a previously identified, unknown M. ***tuberculosis*** sequence. MO-6 was found to show some homology to M. ***tuberculosis*** heat shock protein 65. MO-8, MO-9, MO-10, MO-26 and MO-29 were found to be highly related to each other and to show some homology to M. ***tuberculosis*** dihydrolipamide succinyltransferase. MO-28, MO-31 and MO-32 were found to be identical and to show some homology to a previously identified M. ***tuberculosis*** protein. MO-33 was found to show some homology to a previously identified 14 kDa M. ***tuberculosis*** heat shock protein.

DETD . . . homologies to MO-39. MO-12, MO-13 and MO-19 were found to show some homologies to unknown sequences previously isolated from M. ***tuberculosis*** .

DETD Isolation of DNA Sequences Encoding M. ***tuberculosis*** Antigens by Screening of a Novel Expression Library

DETD [0476] This example illustrates isolation of DNA sequences encoding M. ***tuberculosis*** antigens by screening of a novel expression library with sera from M. ***tuberculosis*** -infected patients that were shown to be unreactive with a panel of the recombinant M. ***tuberculosis*** antigens TbRa11, TbRa3, Tb38-1, TbH4, TbF and 38 kD.

DETD [0477] Genomic DNA from M. ***tuberculosis*** Erdman strain was randomly sheared to an average size of 2 kb, and blunt ended with Kienow polymerase, followed by. . . Wis.) and packaged in vitro using the PhageMaker extract (Novagen). The resulting library was screened with sera from several M. ***tuberculosis*** donors that had been shown to be negative on a panel of previously identified M. ***tuberculosis*** antigens as described above in Example 3B.

DETD . . . 324-326, 328, 330, 332, 334 and 336 were found to show some homology to unknown sequences previously identified in M. ***tuberculosis*** .

DETD Isolation of Soluble M. ***tuberculosis*** Antigens Using Mass Spectrometry

DETD [0479] This example illustrates the use of mass spectrometry to identify soluble M. ***tuberculosis*** antigens.

DETD [0480] In a first approach, M. ***tuberculosis*** culture filtrate was screened by Western analysis using serum from a ***tuberculosis*** -infected individual. The reactive bands were excised from a silver stained gel and the amino acid sequences determined by mass

spectrometry.. . . of this sequence with those in the gene bank revealed homology to the 85b precursor antigen previously identified in M. ***tuberculosis*** .

DETD [0481] In a second approach, the high molecular weight region of M. ***tuberculosis*** culture supernatant was studied. This area may contain immunodominant antigens which may be useful in the diagnosis of M. ***tuberculosis*** infection. Two known monoclonal antibodies, IT42 and IT57 (available from the Center for Disease Control, Atlanta, Ga.), show reactivity by. . . the antigens remains unknown. In addition, unknown high-molecular weight proteins have been described as containing a surrogate marker for M. ***tuberculosis*** infection in HIV-positive individuals (Jnl. Infect. Dis.. 1 76:133-143. 1997).

DETD . . . phosphoenolpyruvate kinase. The two sequences isolated from spot 2 were determined to be from two DNAks, previously identified in M. ***tuberculosis*** as heat shock proteins. Spot 4 was determined to be the previously identified M. ***tuberculosis*** protein Kat G. To the best of the inventors' knowledge, neither PcK-1 nor the two DNAks have previously been shown to have utility in the diagnosis of M. ***tuberculosis*** infection.

DETD Use of Representative Antigens for Serodiagnosis of ***Tuberculosis***

DETD . . . ELISA reactivity of two recombinant antigens isolated using method A in Example 3 (TbRa3 and TbRa9) with sera from M. ***tuberculosis*** positive and negative patients. The reactivity of these antigens is compared to that of bacterial lysate isolated from M. ***tuberculosis*** strain H37Ra (Difco, Detroit, Mich.). In both cases, the recombinant antigens differentiated positive from negative sera. Based on cut-off values. . .

DETD [0492] The reactivity of four antigens (TbRa3, TbRa9, TbH4 and TbH12) with sera from a group of M. ***tuberculosis*** infected patients with differing reactivity in the acid fast stain of sputum (Smithwick and David, Tubercle 52:226, 1971) was also examined, and compared to the reactivity of M. ***tuberculosis*** lysate and the 38 kD antigen. The results are presented in Table 3, below:

TABLE 3

REACTIVITY OF ANTIGENS WITH SERA FROM M. ***TUBERCULOSIS*** PATIENTS

Patient	Acid Fast Sputum	ELISA Values Lysate	38 kD	TbRa9	TbH12	TbH4	TbRa3
Tb01B93I-2	++++	1.853	0.634	0.998	1.022	1.030	1.314

Tb01B93I-19. . .

DETD . . . sensitivity of 27 out of 27, indicating that these antigens should complement each other in the serological detection of M. ***tuberculosis*** infection. In addition, several of the recombinant antigens detected positive sera that were not detected using the 38 kD antigen,. . .

DETD [0494] The reactivity of the recombinant antigen TbRa11 with sera from M. ***tuberculosis*** patients shown to be negative for the 38 kD antigen, as well as with sera from PPD positive and normal. . .

DETD . . . After washing, the assay was developed with TMB substrate as described above. The reactivity of TbRa2A with sera from M. ***tuberculosis*** patients and normal donors in shown in Table 4. The

mean value for reactivity of TbRa2A with sera from of M. ***tuberculosis*** patients was 0.444 with a standard deviation of 0.309. The mean for reactivity with sera from normal donors was 0.109. . . that the TbRa2A antigen was capable of detecting sera in this category.

TABLE 4

REACTIVITY OF TBRA2A WITH SERA FROM M. ***TUBERCULOSIS*** PATIENTS AND FROM NORMAL DONORS

Serum ID	Status	OD 450
Tb85	TB	0.680
Tb86	TB	0.450
Tb87	TB	0.263
Tb88	TB. . .	

DETD [0496] The reactivity of the recombinant antigen (g) (SEQ ID NO: 60) with sera from M. ***tuberculosis*** patients and normal donors was determined by ELISA as described above. FIG. 8 shows the results of the titration of antigen (g) with four M. ***tuberculosis*** positive sera that were all reactive with the 38 kD antigen and with four donor sera All four positive sera. . .

DETD [0497] The reactivity of the recombinant antigen TbH-29 (SEQ ED NO: 137) with sera from M. ***tuberculosis*** patients, PPD positive donors and normal donors was determined by indirect ELISA as described above. The results are shown in FIG. 9. TbH-29 detected 30 out of 60 M. ***tuberculosis*** sera, 2 out of 8 PPD positive sera and 2 out of 27 normal sera.

DETD . . . results of ELISA tests (both direct and indirect) of the antigen TbH-33 (SEQ ID NO: 140) with sera from M. ***tuberculosis*** patients and from normal donors and with a pool of sera from M. ***tuberculosis*** patients. The mean OD 450 was demonstrated to be higher with sera from M. ***tuberculosis*** patients than from normal donors, with the mean OD 450 being significantly higher in the indirect ELISA than in the direct ELISA. FIG. 11 is a titration curve for the reactivity of recombinant TbH-33 with sera from M. ***tuberculosis*** patients and from normal donors showing an increase in OD 450 with increasing concentration of antigen.

DETD [0499] The reactivity of the recombinant antigens RDIF6, RDEF8 and RDIF10 (SEQ ID NOS: 184-187, respectively) with sera from M. ***tuberculosis*** patients and normal donors was determined by ELISA as described above. RDIF6 detected 6 out of 32 M. ***tuberculosis*** sera and 0 out of 15 normal sera; RDIF8 detected 14 out of 32 M. ***tuberculosis*** sera and 0 out of 15 normal sera; and RDIF10 detected 4 out of 27 M. ***tuberculosis*** sera and 1 out of 15 normal sera. In addition, RDIF10 was found to detect 0 out of 5 sera. .

.

DETD . . . 5, were expressed in E. coli and purified using a hexahistidine tag. The reactivity of these antigens with both M. ***tuberculosis*** positive and negative sera was examined by ELISA as described above. Titration curves showing the reactivity of MO-1, MO-2, MO-4, MO-28 and MO-29 at different solid phase coat levels when tested against four M. ***tuberculosis*** positive sera and four M. ***tuberculosis*** negative sera are shown in FIGS. 12A-E, respectively. Three of the clones, MO-1, MO-2 and MO-29 were further tested on panels of HIV

positive/ ***tuberculosis*** (HIV/TB) positive and extrapulmonary sera. MO-1 detected 3/20 extrapulmonary and 2/38 HMV/TB sera. On the same sera groups, MO-2 detected. . . and 16/38 HIV/TB sera. In addition, MO-1 detected 6/17 sera that had previously been shown only to react with M. ***tuberculosis*** lysate and not with either 38 kD or with other antigens of the subject invention.

DETD Preparation and Characterization of M. ***tuberculosis*** Fusion Proteins

DETD [0511] Genomic M. ***tuberculosis*** DNA was used to PCR full-length TbH4 (FL TbH4) with the primers PDM-157 and PDM-160 (SEQ ID NO: 343 and. . .

DETD Use of M. ***tuberculosis*** Fusion Proteins for Serodiagnosis of ***Tuberculosis***

DETD [0515] The effectiveness of the fusion protein TbRa3-38 kD-Tb38-1, prepared as described above, in the serodiagnosis of ***tuberculosis*** infection was examined by ELISA.

DETD . . . 6, with the fusion protein being coated at 200 ng/well. A panel of sera was chosen from a group of ***tuberculosis*** patients previously shown, either by ELISA or by western blot analysis, to react with each of the three antigens individually. . . demonstrates the activity of all three epitopes in the fusion protein.

TABLE 5

REACTIVITY OF TRI-PEPTIDE FUSION PROTEIN WITH SERA FROM M. ***TUBERCULOSIS*** PATIENTS

Serum ID	Status	ELISA and/or Western Blot Reactivity with Individual proteins 38 kd	Tb38-1. . .	Fusion Recom-binant	Fusion Recom-binant

DETD [0517] The reactivity of the fusion protein TbF-2 with sera from M. ***tuberculosis*** -infected patients was examined by ELISA using the protocol described above. The results of these studies (Table 6) demonstrate that all. . .

DETD SEQUENCE CHARACTERISTICS:
LENGTH: 53 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 141
GGATCCATAT GGGCCATCAT CATCATCATC ACGTGATCGA CATCATCGGG ACC 53

DETD SEQUENCE CHARACTERISTICS:
LENGTH: 42 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR Primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 142

CCTGAATTCA GGCCTCGGTT GCGCCGGCCT CATCTTGAAC GA 42
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 31 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR Primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 143
GGATCCTGCA GGCTCGAAAC CACCGAGCGG T 31
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 31 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 144
CTCTGAATTC AGCGCTGGAA ATCGTCGCGA T 31
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 33 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 145
GGATCCAGCG CTGAGATGAA GACCGATGCC GCT 33
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 33 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 146
GAGAGAATTC TCAGAAGCCC ATTTGCGAGG ACA 33
DETD SEQUENCE CHARACTERISTICS:
LENGTH: 1993 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: DNA (genomic)
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
FEATURE:
NAME/KEY: CDS

LOCATION: 152..1273
SEQUENCE: 147
TGTTCTTCGA CGGCAGGCTG GTGGAGGAAG GGCCCACCGA ACAGCTGTTC TCCTCGCCGA 60
AGCATGCGGA AACCGCCCGA TACGTCGCCG GACTGTCGGG GGACGTCAAG GACGCCAAGC 120
GCGGAAATTG AAGAGCACAG AAAGGTATGG. . .
CLM What is claimed is:

1. A polypeptide comprising an antigenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen has an. . .

2. A polypeptide comprising an immunogenic portion of an M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen has an. . .

3. A polypeptide comprising an antigenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an. . .

4. A polypeptide comprising an antigenic portion of a M. ***tuberculosis*** antigen, or a variant of said antigen that differs only in conservative substitutions and/or modifications, wherein said antigen comprises an. . .

9. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting a biological sample with one or more polypeptides according to any of. . . detecting in the sample the presence of antibodies that bind to at least one of the polypeptides, thereby detecting M. ***tuberculosis*** infection in the biological sample.

10. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting a biological sample with a polypeptide having an N-terminal sequence selected from. . . detecting in the sample the presence of antibodies that bind to at least one of the polypeptides, thereby detecting M. ***tuberculosis*** infection in the biological sample.

11. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting a biological sample with one or more polypeptides encoded by a DNA. . . detecting in the sample the presence of antibodies that bind to at least one of the polypeptides, thereby detecting M. ***tuberculosis*** infection in the biological sample.

. . . of any one of claims 9-11 wherein step (a) additionally comprises contacting the biological sample with a 38 kD M. ***tuberculosis*** antigen and step (b) additionally comprises detecting in the sample the presence of antibodies that bind to the 38 kD M. ***tuberculosis*** antigen.

17. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting the sample with at least two oligonucleotide primers in a polymerase chain. . . (b) detecting in the sample a DNA sequence that amplifies in the presence of the oligonucleotide primers, thereby detecting M. ***tuberculosis*** infection.

19. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting the sample with at least two oligonucleotide primers in a polymerase chain. . . the sample a DNA sequence that amplifies in the presence of the first and second oligonucleotide primers, thereby detecting M. ***tuberculosis*** infection.

22. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting the sample with one or more oligonucleotide probes specific for a DNA. . . claim 5; and (b) detecting in the sample a DNA sequence that hybridizes to the oligonucleotide probe, thereby detecting M. ***tuberculosis*** infection.

24. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting the sample with one or more oligonucleotide probes specific for a DNA. . . and 337; and (b) detecting in the sample a DNA sequence that hybridizes to the oligonucleotide probe, thereby detecting M. ***tuberculosis*** infection.

27. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting the biological sample with a binding agent which is capable of binding. . . 1-4; and (b) detecting in the sample a protein or polypeptide that binds to the binding agent, thereby detecting M. ***tuberculosis*** infection in the biological sample.

28. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting the biological sample with a binding agent which is capable of binding. . . 130; and (b) detecting in the sample a protein or polypeptide that binds to the binding agent, thereby detecting M. ***tuberculosis*** infection in the biological sample.

29. A method for detecting M. ***tuberculosis*** infection in a biological sample, comprising: (a) contacting the biological sample with a binding agent which is capable of binding. . . 337; and (b) detecting in the sample a protein or polypeptide that binds to the binding agent, thereby detecting M. ***tuberculosis*** infection in the biological sample.

53. A fusion protein comprising one or more polypeptides according to any one of claims 1-4 and the M. ***tuberculosis*** antigen 38 kD (SEQ ID NO: 150).

L15 ANSWER 3 OF 7 USPATFULL on STN
AN 2003:190560 USPATFULL
TI Compounds and methods for immunotherapy and diagnosis of ***tuberculosis***
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PI US 6592877 B1 20030715
AI US 1998-72967 19980505 (9)
RLI Continuation-in-part of Ser. No. US 1998-25197, filed on 18 Feb 1998, now abandoned Continuation-in-part of Ser. No. US 1997-942578, filed on 1 Oct 1997, now abandoned Continuation-in-part of Ser. No. US 1997-818112, filed on 13 Mar 1997, now patented, Pat. No. US 6290969 Continuation-in-part of Ser. No. US 730510, now abandoned Continuation-in-part of Ser. No. US 1996-680574, filed on 12 Jul 1996, now abandoned Continuation-in-part of Ser. No. US 1996-659683, filed on 5 Jun 1996, now abandoned Continuation-in-part of Ser. No. US 1996-620874, filed on 22 Mar 1996, now abandoned Continuation-in-part of Ser. No. US 1995-533634, filed on 22 Sep 1995, now abandoned Continuation-in-part of Ser. No. US 1995-523436, filed on 1 Sep 1995, now abandoned
DT Utility
FS GRANTED
EXNAM Primary Examiner: Swartz, Rodney P
LREP Townsend and Townsend and Crew LLP
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 26 Drawing Figure(s); 19 Drawing Page(s)
LN.CNT 8747
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Compounds and methods for inducing protective immunity against ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one immunogenic portion of one or more M. ***tuberculosis*** proteins and DNA molecules encoding such polypeptides. Such compounds may be formulated into vaccines and/or pharmaceutical compositions for immunization against M. ***tuberculosis*** infection, or may be used for the diagnosis of ***tuberculosis*** .
TI Compounds and methods for immunotherapy and diagnosis of ***tuberculosis***
AB Compounds and methods for inducing protective immunity against ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one immunogenic portion of one or more M. ***tuberculosis*** proteins and DNA molecules encoding such polypeptides. Such compounds may be formulated into vaccines and/or pharmaceutical compositions for immunization against M. ***tuberculosis*** infection, or may be used for the diagnosis of ***tuberculosis*** .
SUMM The present invention relates generally to detecting, treating and preventing Mycobacterium ***tuberculosis*** infection. The invention is more particularly related to polypeptides comprising a Mycobacterium ***tuberculosis*** antigen, or a portion or other variant thereof, and the use of such polypeptides for diagnosing and vaccinating against Mycobacterium ***tuberculosis*** infection.
SUMM ***Tuberculosis*** is a chronic, infectious disease, that is

generally caused by infection with Mycobacterium ***tuberculosis*** . It is a major disease in developing countries, as well as an increasing problem in developed areas of the world,. . .

SUMM Although ***tuberculosis*** can generally be controlled using extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease.. . .

SUMM Inhibiting the spread of ***tuberculosis*** requires effective vaccination and accurate, early diagnosis of the disease. Currently, vaccination with live bacteria is the most efficient method. . .

SUMM While macrophages have been shown to act as the principal effectors of M. ***tuberculosis*** immunity, T cells are the predominant inducers of such immunity. The essential role of T cells in protection against M. ***tuberculosis*** infection is illustrated by the frequent occurrence of M. ***tuberculosis*** in AIDS patients, due to the depletion of CD4 T cells associated with human immunodeficiency virus (HIV) infection. Mycobacterium-reactive CD4. . . that 1,25-dihydroxy-vitamin D3, either alone or in combination with IFN-.gamma. or tumor necrosis factor-alpha, activates human macrophages to inhibit M. ***tuberculosis*** infection. Furthermore, it is known that IFN-.gamma. stimulates human macrophages to make 1,25-dihydroxy-vitamin D3. Similarly, IL-12 has been shown to play a role in stimulating resistance to M. ***tuberculosis*** infection. For a review of the immunology of M. ***tuberculosis*** infection see Chan and Kaufmann in ***Tuberculosis*** : Pathogenesis, Protection and Control, Bloom (ed.), ASM Press, Washington, D.C., 1994.

SUMM Accordingly, there is a need in the art for improved vaccines and methods for preventing, treating and detecting ***tuberculosis*** . The present invention fulfills these needs and further provides other related advantages.

SUMM Briefly stated, this invention provides compounds and methods for preventing and diagnosing ***tuberculosis*** . In one aspect, polypeptides are provided comprising an immunogenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. In one embodiment of. . .

SUMM In a related aspect, polypeptides are provided comprising an immunogenic portion of an M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, the antigen having one. . .

SUMM In another embodiment, the soluble M. ***tuberculosis*** antigen comprises an amino acid sequence encoded by a DNA sequence selected from the group consisting of the sequences recited. . .

SUMM In a related aspect, the polypeptides comprise an immunogenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, wherein the antigen comprises. . .

SUMM . . . provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, an inventive polypeptide and a known M. ***tuberculosis*** antigen.

SUMM In further aspects of this invention, methods and diagnostic kits are provided for detecting ***tuberculosis*** in a patient. The methods comprise contacting dermal cells of a patient with one or more of the above polypeptides. . .

SUMM In yet other aspects, methods are provided for detecting ***tuberculosis*** in a patient, such methods comprising contacting

dermal cells of a patient with one or more polypeptides encoded by a. .
.

DRWD . . . D illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a first and a second M. ***tuberculosis*** -immune donor, respectively, by the 14 Kd, 20 Kd and 26 Kd antigens described in Example 1.

DRWD FIG. 2 illustrates the stimulation of proliferation and interferon-.gamma. production in T cells derived from an M. ***tuberculosis*** -immune individual by the two representative polypeptides TbRa3 and TbRa9.

DRWD FIGS. 3A-D illustrate the reactivity of antisera raised against secretory M. ***tuberculosis*** proteins, the known M. ***tuberculosis*** antigen 85b and the inventive antigens Tb38-1 and TbH-9, respectively, with M. ***tuberculosis*** lysate (lane 2), M. ***tuberculosis*** secretory proteins (lane 3), recombinant Tb38-1 (lane 4), recombinant TbH-9 (lane 5) and recombinant 85b (lane 5).

DRWD FIG. 4A illustrates the stimulation of proliferation in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, recombinant TbH-9 and a control antigen, TbRa11.

DRWD FIG. 4B illustrates the stimulation of interferon-.gamma. production in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, PPD and recombinant TbH-9.

DRWD FIGS. 8A and B illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a first M. ***tuberculosis*** -immune individual by the representative polypeptides XP-1, RDIF6, RDIF8, RDIF10 and RDIF11.

DRWD FIGS. 9A and B illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a second M. ***tuberculosis*** -immune individual by the representative polypeptides XP-1, RDIF6, RDIF8, RDIF10 and RDIF11.

DRWD SEQ ID NO: 154 is the DNA sequence of the M. ***tuberculosis*** antigen 38 kD.

DRWD SEQ ID NO: 155 is the amino acid sequence of the M. ***tuberculosis*** antigen 38 kD.

DRWD SEQ ID NO: 343 is the determined amino acid sequence for a M. ***tuberculosis*** 85b precursor homolog

DETD As noted above, the present invention is generally directed to compositions and methods for preventing, treating and diagnosing ***tuberculosis*** . The compositions of the subject invention include polypeptides that comprise at least one immunogenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. Polypeptides within the scope of the present invention include, but are not limited to, immunogenic soluble M. ***tuberculosis*** antigens. A "soluble M. ***tuberculosis*** antigen" is a protein of M. ***tuberculosis*** origin that is present in M. ***tuberculosis*** culture filtrate. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e.,. . . . entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native M. ***tuberculosis*** antigen or may be heterologous, and such sequences may (but need not) be immunogenic.

DETD . . . from the group of T cells, NK cells, B cells and macrophages, where the cells are derived from an M. ***tuberculosis*** -immune individual. Polypeptides comprising at least an immunogenic portion of

one or more M. ***tuberculosis*** antigens may generally be used to detect ***tuberculosis*** or to induce protective immunity against ***tuberculosis*** in a patient.

DETD . . . be identified by evaluating a modified polypeptide for the ability to generate antibodies that detect the presence or absence of ***tuberculosis*** . Such modified sequences may be prepared and tested using, for example, the representative procedures described herein.

DETD . . . polypeptide" is a polypeptide comprising at least one of the above immunogenic portions and one or more additional immunogenic M. ***tuberculosis*** sequences, which are joined via a peptide linkage into a single amino acid chain. The sequences may be joined directly. . .

DETD In general, M. ***tuberculosis*** antigens, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, soluble antigens may be isolated from M. ***tuberculosis*** culture filtrate by procedures known to those of ordinary skill in the art, including anion-exchange and reverse phase chromatography. Purified. . .

DETD . . . vector and expressed in an appropriate host. DNA molecules encoding soluble antigens may be isolated by screening an appropriate M. ***tuberculosis*** expression library with anti-sera (e.g., rabbit) raised specifically against soluble M. ***tuberculosis*** antigens. DNA sequences encoding antigens that may or may not be soluble may be identified by screening an appropriate M. ***tuberculosis*** genomic or cDNA expression library with sera obtained from patients infected with M. ***tuberculosis*** . Such screens may generally be performed using techniques well known to those of ordinary skill in the art, such as. . .

DETD DNA sequences encoding soluble antigens may also be obtained by screening an appropriate M. ***tuberculosis*** cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of. . .

DETD Alternatively, genomic or cDNA libraries derived from M. ***tuberculosis*** may be screened directly using peripheral blood mononuclear cells (PBMCs) or T cell lines or clones derived from one or more M. ***tuberculosis*** -immune individuals. In general, PBMCs and/or T cells for use in such screens may be prepared as described below. Direct library. . . of expressed recombinant proteins for the ability to induce proliferation and/or interferon-.gamma. production in T cells derived from an M. ***tuberculosis*** -immune individual. Alternatively, potential T cell antigens may be first selected based on antibody reactivity, as described above.

DETD . . . cytokine production (i.e., interferon-.gamma. and/or interleukin-12 production) in T cells, NK cells, B cells and/or macrophages derived from an M. ***tuberculosis*** -immune individual. The selection of cell type for use in evaluating an immunogenic response to a antigen will, of course, depend. . . the desired response. For example, interleukin-12 production is most readily evaluated using preparations containing B cells and/or macrophages. An M. ***tuberculosis*** -immune individual is one who is considered to be resistant to the development of ***tuberculosis*** by virtue of having mounted an effective T cell response to M. ***tuberculosis*** (i.e., substantially free of disease symptoms). Such individuals may be identified based on a strongly positive (i.e., greater than about 10 mm diameter induration) intradermal skin test response to

tuberculosis proteins (PPD) and an absence of any signs or symptoms of ***tuberculosis*** disease. T cells, NK cells, B cells and macrophages derived from M. ***tuberculosis*** -immune individuals may be prepared using methods known to those of ordinary skill in the art. For example, a preparation of. . . individual mycobacterial proteins, may be employed. Such T cell clones may be generated by, for example, culturing PBMCs from M. ***tuberculosis*** -immune individuals with mycobacterial proteins for a period of 2-4 weeks. This allows expansion of only the mycobacterial protein-specific T cells,. . . production (i.e., interferon-.gamma. and/or interleukin-12 production) performed using T cells, NK cells, B cells and/or macrophages derived from an M. ***tuberculosis*** -immune individual are considered immunogenic. Such assays may be performed, for example, using the representative procedures described below. Immunogenic portions of. . .

DETD . . . and/or interleukin-12 production) in T cells, NK cells, B cells and/or macrophages derived from at least about 25% of M. ***tuberculosis*** -immune individuals. Among these immunogenic antigens, polypeptides having superior therapeutic properties may be distinguished based on the magnitude of the responses. . . proliferation and/or cytokine production in vitro in cells derived from more than about 25% of individuals that are not M. ***tuberculosis*** -immune, thereby eliminating responses that are not specifically due to M. ***tuberculosis*** -responsive cells. Those antigens that induce a response in a high percentage of T cell, NK cell, B cell and/or macrophage preparations from M. ***tuberculosis*** -immune individuals (with a low incidence of responses in cell preparations from other individuals) have superior therapeutic properties.

DETD Antigens with superior therapeutic properties may also be identified based on their ability to diminish the severity of M. ***tuberculosis*** infection in experimental animals, when administered as a vaccine. Suitable vaccine preparations for use on experimental animals are described in. . .

DETD . . . identified based on the ability to elicit a response in an intradermal skin test performed on an individual with active ***tuberculosis*** , but not in a test performed on an individual who is not infected with M. ***tuberculosis*** . Skin tests may generally be performed as described below, with a response of at least 5 mm induration considered positive.

DETD Portions and other variants of M. ***tuberculosis*** antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally. . .

DETD In certain specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a soluble M. ***tuberculosis*** antigen having one of the following N-terminal sequences, or a variant thereof that differs only in conservative substitutions and/or modifications:

DETD In a further specific embodiment, the subject invention discloses polypeptides comprising at least an immunogenic portion of an M. ***tuberculosis*** antigen having one of the following N-terminal sequences, or a variant thereof that differs only in conservative substitutions and/or modifications:

DETD In other specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a soluble M. ***tuberculosis*** antigen (or a variant of such an antigen) that

comprises one or more of the amino acid sequences encoded by. . .

DETD In further specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a M. ***tuberculosis*** antigen (or a variant of such an antigen), which may or may not be soluble, that comprises one or more. . .

DETD In the specific embodiments discussed above, the M. ***tuberculosis*** antigens include variants that are encoded by DNA sequences which are substantially homologous to one or more of DNA sequences. . .

DETD . . . comprising a first and a second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known M. ***tuberculosis*** antigen, such as the 38 kD antigen described in Andersen and Hansen, Infect. Immun. 57:2481-2488, 1989, (Genbank Accession No. M30046). . .

DETD . . . or more of the above polypeptides or fusion proteins (or DNA molecules encoding such polypeptides) to induce protective immunity against ***tuberculosis*** in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be. . . may be free of detectable disease and/or infection. In other words, protective immunity may be induced to prevent or treat ***tuberculosis*** .

DETD . . . adjuvant or a liposome (into which the polypeptide is incorporated). Such pharmaceutical compositions and vaccines may also contain other M. ***tuberculosis*** antigens, either incorporated into a combination polypeptide or present within a separate polypeptide.

DETD . . . above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known M. ***tuberculosis*** antigen, such as the 38 kD antigen described above. For example, administration of DNA encoding a polypeptide of the present. . .

DETD . . . described above, is capable of raising an immune response in an immunized patient sufficient to protect the patient from M. ***tuberculosis*** infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced in situ. . .

DETD . . . aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune responses, such as lipid A, Bortadella pertussis or Mycobacterium ***tuberculosis*** . Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Freund's Complete Adjuvant (Difco Laboratories) and Merck Adjuvant. .

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DETD In another aspect, this invention provides methods for using one or more of the polypeptides described above to diagnose ***tuberculosis*** using a skin test. As used herein, a "skin test" is any assay performed directly on a patient in which. . .

DETD . . . than about 0.5 cm in diameter, preferably greater than about 1.0 cm in diameter, is a positive response, indicative of ***tuberculosis*** infection, which may or may not be manifested as an active disease.

DETD Purification and Characterization of Polypeptides from M. ***Tuberculosis*** Culture Filtrate

DETD This example illustrates the preparation of M. ***tuberculosis*** soluble polypeptides from culture filtrate. Unless otherwise noted, all percentages in the following example are weight per volume.

DETD M. ***tuberculosis*** (either H37Ra, ATCC No. 25177, or H37Rv, ATCC No. 25618) was cultured in sterile GAS media at 37.degree. C. for. . .

DETD Additional soluble antigens were isolated from M. ***tuberculosis***

culture filtrate as follows. M. ***tuberculosis*** culture filtrate was prepared as described above. Following dialysis against Bis-Tris propane buffer, at pH 5.5, fractionation was performed using. . .

DETD . . . sequences that encode the antigens designated as (a), (c), (d) and (g) above were obtained by screening a genomic M. ***tuberculosis*** library using .sup.32P end labeled degenerate oligonucleotides corresponding to the N-terminal sequence and containing M. ***tuberculosis*** codon bias. The screen performed using a probe corresponding to antigen (a) above identified a clone having the sequence provided. . .

DETD . . . was amplified from genomic DNA using the sequence obtained from GENBANK. This sequence was then used to screen the M. ***tuberculosis*** library described below in Example 2 and a full length copy of the M. ***tuberculosis*** homologue was obtained (SEQ ID No. 99).

DETD The amino acid sequence for antigen (j) was found to be homologous to a known M. ***tuberculosis*** protein translated from a DNA sequence. To the best of the inventors' knowledge, this protein has not been previously shown. . .

DETD Use of Patient Sera to Isolate M. ***Tuberculosis*** Antigens

DETD This example illustrates the isolation of antigens from M. ***tuberculosis*** lysate by screening with serum from M. ***tuberculosis*** -infected individuals.

DETD Dessicated M. ***tuberculosis*** H37Ra (Difco Laboratories) was added to a 2% NP40 solution, and alternately homogenized and sonicated three times. The resulting suspension. . . Centriprep 10 (Amicon, Beverley, Mass.) and then screened by Western blot for serological activity using a serum pool from M. ***tuberculosis*** -infected patients which was not immunoreactive with other antigens of the present invention.

DETD A DNA sequence that encodes the antigen designated as (m) above was obtained by screening a genomic M. ***tuberculosis*** Erdman strain library using labeled degenerate oligonucleotides corresponding to the N-terminal sequence of SEQ ID NO:137. A clone was identified. . . NO: 204. Comparison of these sequences with those in the genebank revealed some similarity to sequences previously identified in M. ***tuberculosis*** and M. bovis.

DETD Preparation of DNA Sequences Encoding M. ***Tuberculosis*** Antigens

DETD This example illustrates the preparation of DNA sequences encoding M. ***tuberculosis*** antigens by screening a M. ***tuberculosis*** expression library with sera obtained from patients infected with M. ***tuberculosis*** , or with anti-sera raised against soluble M. ***tuberculosis*** antigens.

DETD A. Preparation of M. ***Tuberculosis*** Soluble Antigens Using Rabbit Anti-sera Raised Against M. ***Tuberculosis*** Supernatant

DETD Genomic DNA was isolated from the M. ***tuberculosis*** strain H37Ra. The DNA was randomly sheared and used to construct an expression library using the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Rabbit anti-sera was generated against secretory proteins of the M. ***tuberculosis*** strains H37Ra, H37Rv and Erdman by immunizing a rabbit with concentrated supernatant of the M ***tuberculosis*** cultures. Specifically, the rabbit was first immunized subcutaneously with 200 .mu.g of protein antigen in a total volume of 2. . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the

nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD Thirty two clones were purified. Of these, 25 represent sequences that have not been previously identified in human M. ***tuberculosis*** . Recombinant antigens were expressed and purified antigens used in the immunological analysis described in Example 1. Proteins were induced by. . .

DETD . . . ID Nos. 76, 68, 70, 75) show some homology to sequences previously identified in Mycobacterium leprae but not in M. ***tuberculosis*** . TbRA2A was found to be a lipoprotein, with a six residue lipidation sequence being located adjacent to a hydrophobic secretory sequence. TbRA11, TbRA26, TbRA28 and TbDPEP (SEQ ID Nos.: 65, 73, 74, 53) have been previously identified in M. ***tuberculosis*** . No significant homologies were found to TbRA1, TbRA3, TbRA4, TbRA9, TbRA10, TbRA13, TbRA17, TbRa19, TbRA29, TbRA32, TbRA36 and the overlapping. . .

DETD . . . results of PBMC proliferation and interferon-.gamma. assays performed on representative recombinant antigens, and using T-cell preparations from several different M. ***tuberculosis*** -immune patients, are presented in Tables 2 and 3, respectively.

DETD These results indicate that these soluble antigens can induce proliferation and/or interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** -immune individual.

DETD B. Use of Sera from Patients Having Pulmonary or Pleural ***Tuberculosis*** to Identify DNA Sequences Encoding M. ***Tuberculosis*** Antigens

DETD . . . DNA library described above, and an additional H37Rv library, were screened using pools of sera obtained from patients with active ***tuberculosis*** . To prepare the H37Rv library, M. ***tuberculosis*** strain H37Rv genomic DNA was isolated, subjected to partial Sau3A digestion and used to construct an expression library using the. . . and TbH=high reactivity) in both ELISA and immunoblot format. A fourth pool of sera from seven patients with active pulmonary ***tuberculosis*** was also employed. All of the sera lacked increased reactivity with the recombinant 38 kD M. ***tuberculosis*** H37Ra phosphate-binding protein.

DETD . . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD Thirty two clones were purified. Of these, 31 represented sequences that had not been previously identified in human M. ***tuberculosis*** . Representative sequences of the DNA molecules identified are provided in SEQ ID Nos.: 26-51 and 105. Of these, TbH-8-2 (SEQ.. . . of the open reading frame for the antigen ESAT-6 previously identified in M. bovis (Acc. No. U34848) and in M. ***tuberculosis*** (Sorensen et al., Infec. Immun. 63:1710-1717, 1995).

DETD . . . sequence of Tb38-1F3 is presented in SEQ. ID. NO. 117. A TbH-9 probe identified three clones in the H37Rv library: ***TbH*** - ***9*** - ***FL*** (SEQ. ID NO. 106), which may be the homologue of TbH-9 (R37Ra), TbH-9-1 (SEQ. ID NO. 108), and TbH-9-4 (SEQ.. . .

DETD Further screening of the M. ***tuberculosis*** genomic DNA library, as described above, resulted in the recovery of ten additional reactive clones, representing seven different genes. One. . . determined to be identical to the 14 Kd alpha crystallin heat shock protein previously shown to be present in M. ***tuberculosis*** , and a third was determined to be identical to the antigen TbH-8 described above. The

determined DNA sequences for the. . . contains the reactive open reading frame), although the 3' end of TbH-29 was found to be identical to the M. ***tuberculosis*** cosmid Y227. TbH-32 and TbH-33 were found to be identical to the previously identified M. ***tuberculosis*** insertion element IS6110 and to the M. ***tuberculosis*** cosmid Y50, respectively. No significant homologies to TbH-30 were found.

DETD . . . and uninduced lysates were run in duplicate on SDS-PAGE and transferred to nitrocellulose filters. Filters were reacted with human M. ***tuberculosis*** sera (1:200 dilution) reactive with TbH and a rabbit sera (1:200 or 1:250 dilution) reactive with the N-terminal 4 Kd. . .

DETD Positive reaction of the recombinant human M. ***tuberculosis*** antigens with both the human M. ***tuberculosis*** sera and anti-lacZ sera indicate that reactivity of the human M. ***tuberculosis*** sera is directed towards the fusion protein. Antigens reactive with the anti-lacZ sera but not with the human M. ***tuberculosis*** sera may be the result of the human M. ***tuberculosis*** sera recognizing conformational epitopes, or the antigen-antibody binding kinetics may be such that the 2 hour sera exposure in the. . .

DETD These results indicate that both the inventive M. ***tuberculosis*** antigens and ESAT-6 can induce proliferation and/or interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** -immune individual. To the best of the inventors' knowledge, ESAT-6 has not been previously shown to stimulate human immune responses

DETD . . . help to localize T-cell epitopes within Tb38-1 capable of inducing proliferation and interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** immune individual.

DETD Studies were undertaken to determine whether the antigens TbH-9 and Tb38-1 represent cellular proteins or are secreted into M. ***tuberculosis*** culture media. In the first study, rabbit sera were raised against A) secretory proteins of M. ***tuberculosis*** , B) the known secretory recombinant M. ***tuberculosis*** antigen 85b, C) recombinant Tb38-1 and D) recombinant TbH-9, using protocols substantially the same as that as described in Example 3A. Total M. ***tuberculosis*** lysate, concentrated supernatant of M. ***tuberculosis*** cultures and the recombinant antigens 85b, TbH-9 and Tb38-1 were resolved on denaturing gels, immobilized on nitrocellulose membranes and duplicate. . .

DETD . . . FIGS. 3A-D, respectively, wherein the lane designations are as follows: 1) molecular weight protein standards; 2) 5 .mu.g of M. ***tuberculosis*** lysate; 3) 5 .mu.g secretory proteins; 4) 50 ng recombinant Tb38-1; 5) 50 ng recombinant TbH-9; and 6) 50 ng. . . by an arrow). These results demonstrate that Tb38-1 and TbH-9 are intracellular antigens and are not actively secreted by M. ***tuberculosis*** .

DETD . . . an intracellular antigen was confirmed by determining the reactivity of TbH-9-specific human T cell clones to recombinant TbH-9, secretory M. ***tuberculosis*** proteins and PPD. A TbH-9-specific T cell clone (designated 131TbH-9) was generated from PBMC of a healthy PPD-positive donor. The proliferative response of 131TbH-9 to secretory proteins, recombinant TbH-9 and a control M. ***tuberculosis*** antigen, TbRa11, was determined by measuring uptake of tritiated thymidine, as described in Example 1. As shown in FIG. 4A, the clone

131TbH-9 responds specifically to TbH-9, showing that TbH-9 is not a significant component of M. ***tuberculosis*** secretory proteins. FIG. 4B shows the production of IFN-.gamma. by a second TbH-9-specific T cell clone (designated PPD 800-10) prepared. . . cell clone with secretory proteins, PPD or recombinant TbH-9. These results further confirm that TbH-9 is not secreted by M. ***tuberculosis*** .

DETD C. Use of Sera from Patients Having Extrapulmonary ***Tuberculosis*** to Identify DNA Sequences Encoding M. ***Tuberculosis*** Antigens

DETD Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). The resulting library was screened using pools of sera obtained from individuals with extrapulmonary ***tuberculosis*** , as described above in Example 3B, with the secondary antibody being goat anti-human IgG+A+M (H+L) conjugated with alkaline phosphatase.

DETD . . . the exception of the 3' ends of XP2 and XP6 which were found to bear some homology to known M. ***tuberculosis*** cosmids. The DNA sequences for XP27 and XP36 are shown in SEQ ID Nos.: 163 and 164, respectively, with the. . .

DETD . . . described herein, recombinant XP1 was found to stimulate cell proliferation and IFN-.gamma. production in T cells isolated from an M. ***tuberculosis*** -immune donors.

DETD D. Use of a Lysate Positive Serum Pool from Patients Having ***Tuberculosis*** to Identify DNA Sequences Encoding M. ***Tuberculosis*** Antigens

DETD Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda Screen expression system (Novagen, Madison, Wis.), as described below in Example 6. Pooled serum obtained from M. ***tuberculosis*** -infected patients and that was shown to react with M. ***tuberculosis*** lysate but not with the previously expressed proteins 38 kD, Tb38-1, TbRa3, TbH4, DPEP and TbRa11, was used to screen. . .

DETD . . . SEQ ID NO: 257-260. The remaining seventeen clones were found to show similarities to unknown sequences previously identified in M. ***tuberculosis*** . The determined 5' CDNA sequences for sixteen of these clones (hereinafter referred to as LSER-1, LSER-3, LSER-4, LSER-5, LSER-6, LSER-8,. . .

DETD E. Preparation of M. ***Tuberculosis*** Soluble Antigens Using Rabbit Anti-Sera Raised Against M. ***Tuberculosis*** Fractionated Proteins

DETD M. ***tuberculosis*** lysate was prepared as described above in Example 2. The resulting material was fractionated by HPLC and the fractions screened by Western blot for serological activity with a serum pool from M. ***tuberculosis*** -infected patients which showed little or no immunoreactivity with other antigens of the present invention. Rabbit anti-sera was generated against the most reactive fraction using the method described in Example 3A . The anti-sera was used to screen an M. ***tuberculosis*** Erdman strain genomic DNA expression library prepared as described above. Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones determined.

DETD . . . Of these, one was found to be TbRa35, described above, and one was found to be the previously identified M. ***tuberculosis***

antigen, HSP60. Of the remaining eight clones, seven (hereinafter referred to as RDIF2, RDIF5, RDIF8, RDIF10, RDIF 11 and RDIF12) were found to bear some similarity to previously identified M. ***tuberculosis*** sequences. The determined DNA sequences for RDIF2, RDIF5, RDIF8, RDIF10 and RDIF11 are provided in SEQ ID Nos.: 189-193, respectively,. . .

DETD . . . 8A-B and 9A-B, these antigens were found to stimulate cell proliferation and IFN-.gamma. production in T cells isolated from M. ***tuberculosis*** -immune donors.

DETD An M. ***tuberculosis*** polypeptide was isolated from tuberculin purified protein derivative (PPD) as follows.

DETD . . . F. et al., Tuberculin purified protein derivative. Preparation and analyses of a large quantity for standard. The American Review of ***Tuberculosis*** 44:9-25, 1941).

DETD M. ***tuberculosis*** Rv strain was grown for 6 weeks in synthetic medium in roller bottles at 37.degree. C. Bottles containing the bacterial. . .

DETD Six fractions were collected, dried, suspended in PBS and tested individually in M. ***tuberculosis*** -infected guinea pigs for induction of delayed type hypersensitivity (DTH) reaction. One fraction was found to induce a strong DTH reaction. . . 80 .mu.l/minute. Eluent was monitored at 215 nm. Eight fractions were collected and tested for induction of DTH in M. ***tuberculosis*** -infected guinea pigs. One fraction was found to induce strong DTH of about 16 mm induration. The other fractions did not. . .

DETD . . . were isolated and found to have the sequences shown in SEQ ID Nos.: 130-133. A subsequent search of the M. ***tuberculosis*** genome database released by the Institute for Genomic Research revealed a match of the DPPD partial amino acid sequence with a sequence present within the M. ***tuberculosis*** cosmid MTY21C12. An open reading frame of 336 bp was identified. The full-length DNA sequence for DPPD is provided in. . .

DETD Use of Sera from ***Tuberculosis*** -Infected Monkeys to Identify DNA Sequences Encoding M. ***Tuberculosis*** Antigens

DETD Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Serum samples were obtained from a cynomolgous monkey 18, 33, 51 and 56 days following infection with M. ***tuberculosis*** Erdman strain. These samples were pooled and used to screen the M. ***tuberculosis*** genomic DNA expression library using the procedure described above in Example 3C. Twenty clones were purified. The determined 5' DNA. . .

DETD . . . MO-35 were found to show a high degree of relatedness and showed some homology to a previously identified unknown M. ***tuberculosis*** sequence and to cosmid MTCI237. MO-2 was found to show some homology to aspartokinase from M. ***tuberculosis*** . Clones MO-3, MO-7 and MO-27 were found to be identical and to show a high degree of relatedness to MO-5. All four of these clones showed some homology to M. ***tuberculosis*** heat shock protein 70. MO-27 was found to show some homology to M. ***tuberculosis*** cosmid MTCY339. MO-4 and MO-34 were found to show some homology to cosmid SCY21B4 and M. smegmatis integration host factor, and were both found to show some homology to a previously identified, unknown M. ***tuberculosis*** sequence. MO-6 was found to show some homology to M. ***tuberculosis*** heat shock protein 65. MO-8, MO-9, MO-10, MO-26 and

MO-29 were found to be highly related to each other and to show some homology to M. ***tuberculosis*** dihydrolipamide succinyltransferase. MO-28, MO-31 and MO-32 were found to be identical and to show some homology to a previously identified M. ***tuberculosis*** protein. MO-33 was found to show some homology to a previously identified 14 kDa M. ***tuberculosis*** heat shock protein.

DETD . . . to MO-39. MO-1 2, MO-13 and MO-19 were found to show some homologies to unknown sequences previously isolated from M. ***tuberculosis*** .

DETD Isolation of DNA Sequences Encoding M. ***Tuberculosis*** Antigens by Screening of a Novel Expression Library

DETD This example illustrates isolation of DNA sequences encoding M. ***tuberculosis*** antigens by screening of a novel expression library with sera from M. ***tuberculosis*** -infected patients that were shown to be unreactive with a panel of the recombinant M. ***tuberculosis*** antigens TbRa11, TbRa3, Tb38-1, TbH4, TbF and 38 kD.

DETD Genomic DNA from M. ***tuberculosis*** Erdman strain was randomly sheared to an average size of 2 kb, and blunt ended with Klenow polymerase, followed by. . . Wis.) and packaged in vitro using the PhageMaker extract (Novagen). The resulting library was screened with sera from several M. ***tuberculosis*** donors that had been shown to be negative on a panel of previously identified M. ***tuberculosis*** antigens as described above in Example 3B.

DETD . . . 330-332, 334, 336, 338, 340 and 342 were found to show some homology to unknown sequences previously identified in M. ***tuberculosis*** .

DETD Isolation of Soluble M. ***Tuberculosis*** Antigens Using Mass Spectrometry

DETD This example illustrates the use of mass spectrometry to identify soluble M. ***tuberculosis*** antigens.

DETD In a first approach, M. ***tuberculosis*** culture filtrate was screened by Western analysis using serum from a ***tuberculosis*** -infected individual. The reactive bands were excised from a silver stained gel and the amino acid sequences determined by mass spectrometry.. . . of this sequence with those in the gene bank revealed homology to the 85b precursor antigen previously identified in M. ***tuberculosis*** .

DETD In a second approach, the high molecular weight region of M. ***tuberculosis*** culture supernatant was studied. This area may contain immunodominant antigens which may be useful in the diagnosis of M. ***tuberculosis*** infection. Two known monoclonal antibodies, IT42 and IT57 (available from the Center for Disease Control, Atlanta, Ga.), show reactivity by. . . the antigens remains unknown. In addition, unknown high-molecular weight proteins have been described as containing a surrogate marker for M. ***tuberculosis*** infection in HIV-positive individuals (Jnl. Infect. Dis., 176:133-143, 1997). To determine the identity of these antigens, two-dimensional gel electrophoresis and. . .

DETD . . . phosphoenolpyruvate kinase. The two sequences isolated from spot 2 were determined to be from two DNAks, previously identified in M. ***tuberculosis*** as heat shock proteins. Spot 4 was determined to be the previously identified M. ***tuberculosis*** protein Kat G. To the best of the inventors' knowledge, neither PcK-1 nor the two DNAks

have previously been shown to have utility in the diagnosis of M. ***tuberculosis*** infection.
DETD Use of Representative Antigens for Diagnosis of ***Tuberculosis***
DETD This example illustrates the effectiveness of several representative polypeptides in skin tests for the diagnosis of M. ***tuberculosis*** infection.
DETD . . . 20 individuals tested, 2 were PPD negative and 18 were PPD positive. Of the PPD positive individuals, 3 had active ***tuberculosis*** , 3 had been previously infected with ***tuberculosis*** and 9 were healthy. In a second study, 13 PPD positive individuals were tested with 0.1 .mu.g TbRa11 in either. . .
DETD Preparation and Characterization of M. ***Tuberculosis*** Fusion Proteins
DETD The reactivity of the fusion protein TbF-2 with sera from M. ***tuberculosis*** -infected patients was examined by ELISA using the protocol described above. The results of these studies (Table 11) demonstrate that all. . .
DETD Genomic M. ***tuberculosis*** DNA was used to PCR full-length TbH4 (FL TbH4) with the primers PDM-157 and PDM-160 (SEQ ID NO: 348 and. .
.
DETD . . . Tyr
100
SEQUENCE CHARACTERISTICS:
LENGTH: 53 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 146
GGATCCATAT GGGCCATCAT CATCATCATC ACGTGATCGA CATCATCGGG ACC 53
SEQUENCE CHARACTERISTICS:
LENGTH: 42 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR Primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 147
CCTGAATTCA GGCCTCGGTT GCGCCGGCCT CATCTTGAAC GA 42
SEQUENCE CHARACTERISTICS:
LENGTH: 31 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR Primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 148
GGATCCTGCA GGCTCGAAAC CACCGAGCGG T 31

SEQUENCE CHARACTERISTICS:
LENGTH: 31 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 149
CTCTGAATTC AGCGCTGGAA ATCGTCGCGA T 31
SEQUENCE CHARACTERISTICS:
LENGTH: 33 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 150
GGATCCAGCG CTGAGATGAA GACCGATGCC GCT 33
SEQUENCE CHARACTERISTICS:
LENGTH: 33 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 151
GAGAGAATTC TCAGAAGCCC ATTTGCGAGG ACA 33
SEQUENCE CHARACTERISTICS:
LENGTH: 1993 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: DNA (genomic)
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
FEATURE:
NAME/KEY: CDS
LOCATION: 152..1273
SEQUENCE: 152
TGTTCTTCGA CGGCAGGCTG GTGGAGGAAG GGCCCACCGA ACAGCTGTTC TCCTCGCCGA 60
AGCATGCGGA AACCGCCCGA TACGTCGCCG GACTGTCGGG GGACGTCAAG GACGCCAAGC 120
GCGGAAATTG AAGAGCACAG AAAGGTATGG C GTG AAA. . .
CLM What is claimed is:

1. An isolated fusion protein comprising four polypleptides, wherein the first polypeptide comprises a M. ***tuberculosis*** antigen having an amino acid sequence of TbRa3 (SEQ ID NO:77) or an immunogenic portion thereof; the second polypeptide comprises a M. ***tuberculosis*** antigen having an amino acid sequence of Tb38-1 (SEQ ID NO:88) or an immunogenic portion thereof; the third polypeptide comprises a M.

tuberculosis antigen having an amino acid sequence of TbH4 (SEQ ID NO:89) or an immunogenic portion thereof; and the fourth polypeptide comprises a M. ***tuberculosis*** antigen having an amino acid sequence of 38 kD (SEQ ID NO:155) or an immunogenic portion thereof.

L15 ANSWER 4 OF 7 USPATFULL on STN
AN 2002:254057 USPATFULL
TI Compounds and methods for diagnosis of ***tuberculosis***
IN Reed, Steven G., Bellevue, WA, United States
Skeiky, Yasir A. W., Seattle, WA, United States
Dillon, Davin C., Redmond, WA, United States
Campos-Neto, Antonio, Bainbridge Island, WA, United States
Houghton, Raymond, Bothell, WA, United States
Vedvick, Thomas S., Federal Way, WA, United States
Twardzik, Daniel R., Bainbridge Island, WA, United States
Lodes, Michael J., Seattle, WA, United States
Hendrickson, Ronald C., Seattle, WA, United States
PA Corixa Corporation, Seattle, WA, United States (U.S. corporation)
PI US 6458366 B1 20021001
AI US 1998-72596 19980505 (9)
RLI Continuation-in-part of Ser. No. US 1998-24753, filed on 18 Feb 1998, now abandoned Continuation-in-part of Ser. No. US 1997-942341, filed on 1 Oct 1997, now abandoned Continuation-in-part of Ser. No. US 1997-818111, filed on 13 Mar 1997 Continuation-in-part of Ser. No. US 1996-729622, filed on 11 Oct 1996, now abandoned Continuation-in-part of Ser. No. US 1996-680574, filed on 12 Jul 1996, now abandoned Continuation-in-part of Ser. No. US 1996-658800, filed on 5 Jun 1996, now abandoned Continuation-in-part of Ser. No. US 1996-620280, filed on 22 Mar 1996, now abandoned Continuation-in-part of Ser. No. US 1995-532136, filed on 22 Sep 1995, now abandoned Continuation of Ser. No. US 1995-523435, filed on 1 Sep 1995, now abandoned
PRAI WO 1996-US14675 19960830
DT Utility
FS GRANTED
EXNAM Primary Examiner: Swartz, Rodney P.
LREP Townsend & Townsend & Crew, LLP
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 23 Drawing Figure(s); 19 Drawing Page(s)
LN.CNT 8789
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Compounds and methods for diagnosing ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of one or more M. ***tuberculosis*** proteins, and DNA sequences encoding such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of M. ***tuberculosis*** infection in patients and biological samples. Antibodies directed against such polypeptides are also provided.
TI Compounds and methods for diagnosis of ***tuberculosis***
AB Compounds and methods for diagnosing ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of one or more M. ***tuberculosis*** proteins, and DNA sequences encoding such polypeptides. Diagnostic kits

containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of M. ***tuberculosis*** infection in patients and biological samples. Antibodies directed against such polypeptides are also provided.

SUMM The present invention relates generally to the detection of Mycobacterium ***tuberculosis*** infection. The invention is more particularly related to polypeptides comprising a Mycobacterium ***tuberculosis*** antigen, or a portion or other variant thereof, and the use of such polypeptides for the serodiagnosis of Mycobacterium ***tuberculosis*** infection.

SUMM ***Tuberculosis*** is a chronic, infectious disease, that is generally caused by infection with Mycobacterium ***tuberculosis*** . It is a major disease in developing countries, as well as an increasing problem in developed areas of the world,. . .

SUMM Although ***tuberculosis*** can generally be controlled using extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease.. . .

SUMM Inhibiting the spread of ***tuberculosis*** will require effective vaccination and accurate, early diagnosis of the disease. Currently, vaccination with live bacteria is the most efficient. . .

SUMM While macrophages have been shown to act as the principal effectors of M. ***tuberculosis*** immunity, T cells are the predominant inducers of such immunity. The essential role of T cells in protection against M. ***tuberculosis*** infection is illustrated by the frequent occurrence of M. ***tuberculosis*** in AIDS patients, due to the depletion of CD4 T cells associated with human immunodeficiency virus (HIV) infection. Mycobacterium-reactive CD4. . . that 1,25-dihydroxy-vitamin D3, either alone or in combination with IFN-.gamma. or tumor necrosis factor-alpha, activates human macrophages to inhibit M. ***tuberculosis*** infection. Furthermore, it is known that IFN-.gamma. stimulates human macrophages to make 1,25-dihydroxy-vitamin D3. Similarly, IL-12 has been shown to play a role in stimulating resistance to M. ***tuberculosis*** infection. For a review of the immunology of M. ***tuberculosis*** infection see Chan and Kaufmann, in ***Tuberculosis*** : Pathogenesis, Protection and Control, Bloom (ed.), ASM Press, Washington, D.C., 1994.

SUMM Accordingly, there is a need in the art for improved diagnostic methods for detecting ***tuberculosis*** . The present invention fulfills this need and further provides other related advantages.

SUMM Briefly stated, the present invention provides compositions and methods for diagnosing ***tuberculosis*** . In one aspect, polypeptides are provided comprising an antigenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. In one embodiment of. . .

SUMM In a related aspect, polypeptides are provided comprising an immunogenic portion of an M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, the antigen having one. . .

SUMM In another embodiment, the soluble M. ***tuberculosis*** antigen comprises an amino acid sequence encoded by a DNA sequence selected from the group consisting of the sequences recited. . .

SUMM In a related aspect, the polypeptides comprise an antigenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, wherein

the antigen comprises. . .

SUMM . . . provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, an inventive polypeptide and a known M. ***tuberculosis*** antigen.

SUMM In further aspects of the subject invention, methods and diagnostic kits are provided for detecting ***tuberculosis*** in a patient. The methods comprise: (a) contacting a biological sample with at least one of the above polypeptides; and (b) detecting in the sample the presence of antibodies that bind to the polypeptide or polypeptides, thereby detecting M. ***tuberculosis*** infection in the biological sample. Suitable biological samples include whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine. The. . .

SUMM The present invention also provides methods for detecting M. ***tuberculosis*** infection comprising: (a) obtaining a biological sample from a patient; (b) contacting the sample with at least one oligonucleotide primer. . .

SUMM In a further aspect, the present invention provides a method for detecting M. ***tuberculosis*** infection in a patient comprising: (a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide. . .

SUMM . . . monoclonal, that bind to the polypeptides described above, as well as methods for their use in the detection of M. ***tuberculosis*** infection.

DRWD . . . 1B illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a first and a second M. ***tuberculosis*** -immune donor, respectively, by the 14 Kd, 20 Kd and 26 Kd antigens described in Example 1.

DRWD FIGS. 2A-D illustrate the reactivity of antisera raised against secretory M. ***tuberculosis*** proteins, the known M. ***tuberculosis*** antigen 85b and the inventive antigens Tb38-1 and TbH-9, respectively, with M. ***tuberculosis*** lysate (lane 2), M. ***tuberculosis*** secretory proteins (lane 3), recombinant Tb38-1 (lane 4), recombinant TbH-9 (lane 5) and recombinant 85b (lane 5).

DRWD FIG. 3A illustrates the stimulation of proliferation in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, recombinant TbH-9 and a control antigen, TbRa11.

DRWD FIG. 3B illustrates the stimulation of interferon-.gamma. production in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, PPD and recombinant TbH-9.

DRWD FIG. 4 illustrates the reactivity of two representative polypeptides with sera from M. ***tuberculosis*** -infected and uninfected individuals, as compared to the reactivity of bacterial lysate.

DRWD FIG. 5 shows the reactivity of four representative polypeptides with sera from M. ***tuberculosis*** -infected and uninfected individuals, as compared to the reactivity of the 38 kD antigen.

DRWD FIG. 6 shows the reactivity of recombinant 38 kD and ThRa 11 antigens with sera from M. ***tuberculosis*** patients, PPD positive donors and normal donors.

DRWD FIG. 8 shows the reactivity of the antigen of SEQ ID NO: 60 with sera from M. ***tuberculosis*** patients and normal donors.

DRWD FIG. 9 illustrates the reactivity of the recombinant antigen TbH-29 (SEQ ID NO: 137) with sera from M. ***tuberculosis*** patients, PPD positive donors and normal donors as determined by indirect ELISA.

DRWD FIG. 10 illustrates the reactivity of the recombinant antigen TbH-33 (SEQ ID NO: 140) with sera from M. ***tuberculosis*** patients and

from normal donors, and with a pool of sera from M. ***tuberculosis*** patients, as determined both by direct and indirect ELISA

DRWD . . . 11 illustrates the reactivity of increasing concentrations of the recombinant antigen TbH-33 (SEQ ID NO: 140) with sera from M. ***tuberculosis*** patients and from normal donors as determined by ELISA.

DRWD FIGS. 12A-E illustrate the reactivity of the recombinant antigens MO-1, MO-2, MO-4, MO-28 and MO-29, respectively, with sera from M. ***tuberculosis*** patients and from normal donors as determined by ELISA.

DRWD SEQ ID NO: 149 is the DNA sequence of the M. ***tuberculosis*** antigen 38 kD).

DRWD SEQ ID NO: 150 is the amino acid sequence of the M. ***tuberculosis*** antigen 38 kD.

DRWD SEQ ID NO: 338 is the determined amino acid sequence for a M. ***tuberculosis*** 85b precursor homolog

DETD As noted above, the present invention is generally directed to compositions and methods for diagnosing ***tuberculosis*** . The compositions of the subject invention include polypeptides that comprise at least one antigenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. Polypeptides within the scope of the present invention include, but are not limited to, soluble M. ***tuberculosis*** antigens. A "soluble M. ***tuberculosis*** antigen" is a protein of M. ***tuberculosis*** origin that is present in M. ***tuberculosis*** culture filtrate. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e.,. . . entirely of the antigenic portion, or may contain additional sequences. The additional sequences may be derived from the native M. ***tuberculosis*** antigen or may be heterologous, and such sequences may (but need not) be antigenic.

DETD . . . may or may not be soluble) is a portion that is capable of reacting with sera obtained from an M. ***tuberculosis*** -infected individual (i.e., generates an absorbance reading with sera from infected individuals that is at least three standard deviations above the absorbance obtained with sera from uninfected individuals, in a representative ELISA assay described herein). An "M. ***tuberculosis*** -infected individual" is a human who has been infected with M. ***tuberculosis*** (e.g., has an intradermal skin test response to PPD that is at least 0.5 cm in diameter). Infected individuals may display symptoms of ***tuberculosis*** or may be free of disease symptoms. Polypeptides comprising at least an antigenic portion of one or more M. ***tuberculosis*** antigens as described herein may generally be used, alone or in combination, to detect ***tuberculosis*** in a patient.

DETD . . . be identified by evaluating a modified polypeptide for the ability to generate antibodies that detect the presence or absence of ***tuberculosis*** . Such modified sequences may be prepared and tested using, for example, the representative procedures described herein.

DETD . . . polypeptide" is a polypeptide comprising at least one of the above antigenic portions and one or more additional antigenic M. ***tuberculosis*** sequences, which are joined via a peptide linkage into a single amino acid chain. The sequences may be joined directly. .

.

DETD In general, M. ***tuberculosis*** antigens, and DNA sequences

encoding such antigens, may be prepared using any of a variety of procedures. For example, soluble antigens may be isolated from M. ***tuberculosis*** culture filtrate by procedures known to those of ordinary skill in the art, including anion-exchange and reverse phase chromatography. Purified. . . may then be evaluated for a desired property, such as the ability to react with sera obtained from an M. ***tuberculosis*** -infected individual. Such screens may be performed using the representative methods described herein. Antigens may then be partially sequenced using, for. . .

DETD . . . vector and expressed in an appropriate host. DNA molecules encoding soluble antigens may be isolated by screening an appropriate M. ***tuberculosis*** expression library with anti-sera (e.g., rabbit) raised specifically against soluble M. ***tuberculosis*** antigens. DNA sequences encoding antigens that may or may not be soluble may be identified by screening an appropriate M. ***tuberculosis*** genomic or cDNA expression library with sera obtained from patients infected with M. ***tuberculosis*** . Such screens may generally be performed using techniques well known in the art, such as those described in Sambrook et. . .

DETD DNA sequences encoding soluble antigens may also be obtained by screening an appropriate M. ***tuberculosis*** cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of. . .

DETD . . . antigens described herein are "antigenic." More specifically, the antigens have the ability to react with sera obtained from an M. ***tuberculosis*** -infected individual. Reactivity may be evaluated using, for example, the representative ELISA assays described herein, where an absorbance reading with sera. . .

DETD Antigenic portions of M. ***tuberculosis*** antigens may be prepared and identified using well known techniques, such as those summarized in Paul, Fundamental Immunology, 3d ed.,. . . is substantially similar to that generated by the full length antigen. In other words, an antigenic portion of a M. ***tuberculosis*** antigen generates at least about 20%, and preferably about 100%, of the signal induced by the fill length antigen in. . .

DETD Portions and other variants of M. ***tuberculosis*** antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally. . .

DETD In certain specific embodiments, the subject invention discloses polypeptides comprising at least an antigenic portion of a soluble M. ***tuberculosis*** antigen (or a variant of such an antigen), where the antigen has one of the following N-terminal sequences:

DETD In a further specific embodiment, the subject invention discloses polypeptides comprising at least an immunogenic portion of an M. ***tuberculosis*** antigen having one of the following N-terminal sequences, or a variant thereof that differs only in conservative substitutions and/or modifications:

DETD In other specific embodiments, the subject invention discloses polypeptides comprising at least an antigenic portion of a soluble M. ***tuberculosis*** antigen (or a variant of such an antigen) that comprises one or more of the amino acid sequences encoded by. . .

DETD In further specific embodiments, the subject invention discloses polypeptides comprising at least an antigenic portion of a M. ***tuberculosis*** antigen (or a variant of such an antigen), which may or may not be soluble, that comprises one or more. . .

DETD . . . comprising a first and a second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known M. ***tuberculosis*** antigen, such as the 38 kD antigen described in Andersen and Hansen, Infect. Immun. 57:2481-2488, 1989, (Genbank Accession No. M30046). . .

DETD In another aspect, the present invention provides methods for using the polypeptides described above to diagnose ***tuberculosis*** . In this aspect, methods are provided for detecting M. ***tuberculosis*** infection in a biological sample, using one or more of the above polypeptides, alone or in combination. In embodiments in. . . a predetermined cut-off value. The presence of such antibodies indicates previous sensitization to mycobacterial antigens which may be indicative of ***tuberculosis*** .

DETD . . . using each polypeptide individually to evaluate serum samples obtained from a series of patients known to be infected with M. ***tuberculosis*** . After determining which samples test positive (as described below) with each polypeptide, combinations of two or more polypeptides may be. . . infection in most, or all, of the samples tested. Such polypeptides are complementary. For example, approximately 25-30% of sera from ***tuberculosis*** -infected individuals are negative for antibodies to any single protein, such as the 38 kD antigen mentioned above. Complementary polypeptides may,. . .

DETD . . . (i.e., incubation time) is that period of time that is sufficient to detect the presence of antibody within a M. ***tuberculosis*** -infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that. . .

DETD To determine the presence or absence of anti-M. ***tuberculosis*** antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally. . . general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for ***tuberculosis*** . In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of. . . a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for ***tuberculosis*** .

DETD . . . reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of anti-M. ***tuberculosis*** antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line,. . .

DETD Antibodies may be used in diagnostic tests to detect the presence of M. ***tuberculosis*** antigens using assays similar to those detailed above and other techniques well known to those of skill in the art, thereby providing a method for detecting M. ***tuberculosis*** infection in a patient.

DETD . . . example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify M. ***tuberculosis*** -specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a DNA molecule. . .

DETD . . . art (see, for example, Mullis et al. Ibid; Ehrlich, Ibid). Primers or probes may thus be used to detect M. ***tuberculosis*** -specific sequences in biological samples. DNA probes or primers

comprising oligonucleotide sequences described above may be used alone, in combination with. . .

DETD Purifacation and Characterization of Polypeptides From M. ***Tuberculosis*** Culture Filtrate

DETD This example illustrates the preparation of M. ***tuberculosis*** soluble polypeptides from culture filtrate. Unless otherwise noted, all percentages in the following example are weight per volume.

DETD M. ***tuberculosis*** (either H37Ra, ATCC No. 25177, or H37Rv, ATCC No. 25618) was cultured in sterile GAS media at 37.degree. C. for. . .

DETD Additional soluble antigens were isolated from M. ***tuberculosis*** culture filtrate as follows. M. ***tuberculosis*** culture filtrate was prepared as described above. Following dialysis against Bis-Tris propane buffer, at pH 5.5, fractionation was performed using. . .

DETD DNA sequences that encode the antigens designated as (a), (c), (d) and (g) above were obtained by screening a M. ***tuberculosis*** genomic library using .sup.32P end labeled degenerate oligonucleotides corresponding to the N-terminal sequence and containing M. ***tuberculosis*** codon bias. The screen performed using a probe corresponding to antigen (a) above identified a clone having the sequence provided. . .

DETD . . . was amplified from genomic DNA using the sequence obtained from GENBANK. This sequence was then used to screen an M. ***tuberculosis*** library and a full length copy of the M. ***tuberculosis*** homologue was obtained (SEQ ID NO: 94).

DETD The amino acid sequence for antigen (j) was found to be homologous to a known M. ***tuberculosis*** protein translated from a DNA sequence. To the best of the inventors' knowledge, this protein has not been previously shown. . .

DETD Use of Patient Sera to Isolate M. ***Tuberculosis*** Antigenes

DETD This example illustrates the isolation of antigens from M. ***tuberculosis*** lysate by screening with serum from M. ***tuberculosis*** -infected individuals.

DETD Dessicated M. ***tuberculosis*** H37Ra (Difco Laboratories) was added to a 2% NP40 solution, and alternately homogenized and sonicated three times. The resulting suspension. . . a Centriprep 10 (Amicon, Beverley, Mass.) and screened by Western blot for serological activity using a serum pool from M. ***tuberculosis*** -infected patients which was not immunoreactive with other antigens of the present invention.

DETD A DNA sequence that encodes the antigen designated as (m) above was obtained by screening a genomic M. ***tuberculosis*** Erdman strain library using labeled degenerate oligonucleotides corresponding to the N-terminal sequence of SEQ ID NO:137. A clone was identified. . . NO: 199. Comparison of these sequences with those in the genebank revealed some similarity to sequences previously identified in M. ***tuberculosis*** and M. bovis.

DETD Preparation of Dna Sequences Encoding M. ***Tuberculosis*** Antigens

DETD This example illustrates the preparation of DNA sequences encoding M. ***tuberculosis*** antigens by screening a M. ***tuberculosis*** expression library with sera obtained from patients infected with M. ***tuberculosis*** , or with anti-sera raised against M. ***tuberculosis*** antigens.

DETD A. Preparation of M. ***Tuberculosis*** Soluble Antigens Using Rabbit Anti-Sera Raised Against M. ***Tuberculosis*** Supernant

DETD Genomic DNA was isolated from the M. ***tuberculosis*** strain

H37Ra. The DNA was randomly sheared and used to construct an expression library using the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Rabbit anti-sera was generated against secretory proteins of the M. ***tuberculosis*** strains H37Ra, H37Rv and Erdman by immunizing a rabbit with concentrated supernatant of the M. ***tuberculosis*** cultures. Specifically, the rabbit was first immunized subcutaneously with 200 .mu.g of protein antigen in a total volume of 2. . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD Thirty two clones were purified. Of these, 25 represent sequences that have not been previously identified in M. ***tuberculosis*** . Proteins were induced by IPTG and purified by gel elution, as described in Skeiky et al., J. Exp. Med. 181:1527-1537,. . .

DETD . . . ID NOS: 77, 69, 71, 76) show some homology to sequences previously identified in Mycobacterium leprae but not in M. ***tuberculosis*** . TbRA2A was found to be a lipoprotein, with a six residue lipidation sequence being located adjacent to a hydrophobic secretory sequence. TbRA11, ThRA26, TbRA28 and TbDPEP (SEQ ID NOS: 66, 74, 75, 53) have been previously identified in M. ***tuberculosis*** . No significant homologies were found to TbRA1, TbRA3, ThRA4, TbRA9, ThRA10, TbRA13, TbRA17, TbRA19, ThRA29, TbRA32, ThRA36 and the overlapping. . .

DETD B. Use of Sera From Patients Having Pulmonary or Pleural ***Tuberculosis*** to Identity Dna Sequences Encoding M. ***Tuberculosis*** Antigens

DETD . . . DNA library described above, and an additional H37Rv library, were screened using pools of sera obtained from patients with active ***tuberculosis*** . To prepare the H37Rv library, M. ***tuberculosis*** strain H37Rv genomic DNA was isolated, subjected to partial Sau3A digestion and used to construct an expression library using the. . . and TbH=high reactivity) in both ELISA and immunoblot format. A fourth pool of sera from seven patients with active pulmonary ***tuberculosis*** was also employed. All of the sera lacked increased reactivity with the recombinant 38 kD M. ***tuberculosis*** H37Ra phosphate-binding protein.

DETD . . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD Thirty two clones were purified. Of these, 31 represented sequences that had not been previously identified in human M. ***tuberculosis*** . Representative sequences of the DNA molecules identified are provided in SEQ ID NOS: 26-51 and 100. Of these, TbH-8-2 (SEQ.. . . of the open reading frame for the antigen ESAT-6 previously identified in M. bovis (Acc. No. U34848) and in M. ***tuberculosis*** (Sorensen et al., Infec. Immun. 63:1710-1717, 1995).

DETD . . . sequence of Tb38-1F3 is presented in SEQ. ID. NO. 112. A TbH-9 probe identified three clones in the H37Rv library: ***TbH*** - ***9*** - ***FL*** (SEQ. ID NO. 101), which may be the homologue of TbH-9 (R37Ra), TbH-9-1 (SEQ. ID NO. 103), and TbH-8-2 (SEQ.. . .

DETD Further screening of the M. ***tuberculosis*** genomic DNA library, as described above, resulted in the recovery of ten additional reactive clones, representing seven different genes. One. . . determined to be identical to the 14 Kd alpha crystallin heat shock protein previously shown to be present in M. ***tuberculosis*** , and a third was

determined to be identical to the antigen TbH-8 described above. The determined DNA sequences for the. . . contains the reactive open reading frame), although the 3' end of TbH-29 was found to be identical to the M. ***tuberculosis*** cosmid Y227. TbH-32 and TbH-33 were found to be identical to the previously identified M. ***tuberculosis*** insertion element IS6110 and to the M. ***tuberculosis*** cosmid Y50, respectively. No significant homologies to TbH-30 were found.

DETD . . . and uninduced lysates were run in duplicate on SDS-PAGE and transferred to nitrocellulose filters. Filters were reacted with human M. ***tuberculosis*** sera (1:200 dilution) reactive with TbH and a rabbit sera (1:200 or 1:250 dilution) reactive with the N-terminal 4 Kd. . .

DETD Positive reaction of the recombinant human M. ***tuberculosis*** antigens with both the human M. ***tuberculosis*** sera and anti-lacZ sera indicate that reactivity of the human M. ***tuberculosis*** sera is directed towards the fusion protein. Antigens reactive with the anti-lacZ sera but not with the human M. ***tuberculosis*** sera may be the result of the human M. ***tuberculosis*** sera recognizing conformational epitopes, or the antigen-antibody binding kinetics may be such that the 2 hour sera exposure in the. . .

DETD Studies were undertaken to determine whether the antigens TbH-9 and Tb38-1 represent cellular proteins or are secreted into M. ***tuberculosis*** culture media. In the first study, rabbit sera were raised against A) secretory proteins of M. ***tuberculosis*** , B) the known secretory recombinant M. ***tuberculosis*** antigen 85b, C) recombinant Tb38-1 and D) recombinant TbH-9, using protocols substantially as described in Example 3A. Total M. ***tuberculosis*** lysate, concentrated supernatant of M. ***tuberculosis*** cultures and the recombinant antigens 85b, TbH-9 and Tb38-1 were resolved on denaturing gels, immobilized on nitrocellulose membranes and duplicate. . .

DETD . . . FIGS. 2A-D, respectively, wherein the lane designations are as follows: 1) molecular weight protein standards; 2) 5 .mu.g of M. ***tuberculosis*** lysate; 3) 5 .mu.g secretory proteins; 4) 50 ng recombinant Tb38-1; 5) 50 ng recombinant TbH-9; and 6) 50 ng. . . by an arrow). These results demonstrate that Tb38-1 and TbH-9 are intracellular antigens and are not actively secreted by M. ***tuberculosis*** .

DETD . . . an intracellular antigen was confirmed by determining the reactivity of TbH-9-specific human T cell clones to recombinant TbH-9, secretory M. ***tuberculosis*** proteins and PPD. A TbH-9-specific T cell clone (designated 131TbH-9) was generated from PBMC of a healthy PPD-positive donor. The proliferative response of 131 TbH-9 to secretory proteins, recombinant TbH-9 and a control M. ***tuberculosis*** antigen, TbRa11, was determined by measuring uptake of tritiated thymidine, as described in Example 1. As shown in FIG. 3A, the clone 131TbH-9 responds specifically to TbH-9, showing that TbH-9 is not a significant component of M. ***tuberculosis*** secretory proteins. FIG. 3B shows the production of IFN-.gamma. by a second TbH-9-specific T cell clone (designated PPD 800-10) prepared. . . cell clone with secretory proteins, PPD or recombinant TbH-9. These results further confirm that TbH-9 is not secreted by M. ***tuberculosis*** .

DETD C. Use of Sera From Patients Havings Extrapulmonary ***Tuberculosis***

to Identify Dna Sequences Encoding M. ***Tuberculosis*** Antigens

DETD Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). The resulting library was screened using pools of sera obtained from individuals with extrapulmonary ***tuberculosis*** , as described above in Example 3B, with the secondary antibody being goat anti-human IgG+A+M (H+L) conjugated with alkaline phosphatase.

DETD . . . the exception of the 3' ends of XP2 and XP6 which were found to bear some homology to known M ***tuberculosis*** cosmids. The DNA sequences for XP27 and XP36 are shown in SEQ ID NOS: 158 and 159, respectively, with the. . .

DETD . . . for purification. Recombinant two was found to stimulate cell proliferation and IFN-.gamma. production in T cells isolated from an M. ***tuberculosis*** -immune donors.

DETD D. Use of a Lysate Positive Serum Pool From Patients Having ***Tuberculosis*** to Identity Dna Sequences Encoding M. ***Tuberculosis*** Antigens

DETD Genomic DNA was isolated from M. ***tuberculosis*** Erdinan strain, randomly sheared and used to construct an expression library employing the Lambda Screen expression system (Novagen, Madison, Wis.), as described below in Example 6. Pooled serum obtained from M. ***tuberculosis*** -infected patients and that was shown to react with M. ***tuberculosis*** lysate but not with the previously expressed proteins 38kD, Th38-1, TbRa3, TbH4, DPEP and TbRa11, was used to screen the. . .

DETD . . . SEQ ID NO: 252-255. The remaining seventeen clones were found to show similarities to unknown sequences previously identified in M. ***tuberculosis*** . The determined 5' cDNA sequences for sixteen of these clones (hereinafter referred to as LSER-1, LSER-3, LSER-4, LSER-5, LSER-6, LSER-8,. . .

DETD E. Preparation of M. ***Tuberculosis*** Soluble Antigens Using Rabbit Anti-Sera Raised Against M. ***Tuberculosis*** Fractionated Proteins

DETD M. ***tuberculosis*** lysate was prepared as described above in Example 2. The resulting material was fractionated by HPLC and the fractions screened by Western blot for serological activity with a serum pool from M. ***tuberculosis*** -infected patients which showed little or no immunoreactivity with other antigens of the present invention. Rabbit anti-sera was generated against the most reactive fraction using the method described in Example 3A . The anti-sera was used to screen an M. ***tuberculosis*** Erdman strain genomic DNA expression library prepared as described above. Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones determined.

DETD . . . Of these, one was found to be TbRa35, described above, and one was found to be the previously identified M. ***tuberculosis*** antigen, HSP60. Of the remaining eight clones, six (hereinafter referred to as RDIF2, RDIF5, RDIF8, RDIF10, RDIF11 and RDIF12) were found to bear some similarity to previously identified M. ***tuberculosis*** sequences. The determined DNA sequences for RDIF2, RDIF5, RDIF8, RDIF10 and RDIF1 1 are provided in SEQ ID NOS: 184-188,. . .

DETD . . . as described above. These antigens were found to stimulate cell proliferation and IFN-.gamma. production in T cells isolated from M.

tuberculosis -immune donors.

DETD An M. ***tuberculosis*** polypeptide was isolated from tuberculin purified protein derivative (PPD) as follows.

DETD . . . F. et al., Tuberculin purified protein derivative. Preparation and analyses of a large quantity for standard. The American Review of ***Tuberculosis*** 44:9-25, 1941). M. ***tuberculosis*** Rv strain was grown for 6 weeks in synthetic medium in roller bottles at 37.degree. C. Bottles containing the bacterial. . .

DETD Six fractions were collected, dried, suspended in PBS and tested individually in M. ***tuberculosis*** -infected guinea pigs for induction of delayed type hypersensitivity (DTH) reaction. One fraction was found to induce a strong DTH reaction. . . 80 .mu.l/minute. Eluent was monitored at 215 nm. Eight fractions were collected and tested for induction of DTH in M. ***tuberculosis*** -infected guinea pigs. One fraction was found to induce strong DTH of about 16 mm induration. The other fractions did not. . .

DETD . . . were isolated and found to have the sequences shown in SEQ ID NOS: 125-128. A subsequent search of the M. ***tuberculosis*** genome database released by the Institute for Genomic Research revealed a match of the DPPD partial amino acid sequence with a sequence present within the M. ***tuberculosis*** cosmid MTY21C 12. An open reading frame of 336 bp was identified. The full-length DNA sequence for DPPD is provided. . .

DETD Use of Sera From ***Tuberculosis*** -Infected Monkeys to Identify Dna Sequences Encoding M. ***Tuberculosis*** Antigens

DETD Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Serum samples were obtained from a cynomolgous monkey 18, 33, 51 and 56 days following infection with M. ***tuberculosis*** Erdman strain. These samples were pooled and used to screen the M. ***tuberculosis*** genomic DNA expression library using the procedure described above in Example 3C.

DETD . . . MO-35 were found to show a high degree of relatedness and showed some homology to a previously identified unknown M. ***tuberculosis*** sequence and to cosmid MTCI237. MO-2 was found to show some homology to aspartokinase from M. ***tuberculosis*** . Clones MO-3, MO-7 and MO-27 were found to be identical and to show a high degree of relatedness to MO-5. All four of these clones showed some homology to M. ***tuberculosis*** heat shock protein 70. MO-27 was found to show some homology to M. ***tuberculosis*** cosmid MTCY339. MO-4 and MO-34 were found to show some homology to cosmid SCY21B4 and M smegmatis integration host factor, and were both found to show some homology to a previously identified, unknown M. ***tuberculosis*** sequence. MO-6 was found to show some homology to M. ***tuberculosis*** heat shock protein 65. MO-8, MO-9, MO-10, MO-26 and MO-29 were found to be highly related to each other and to show some homology to M. ***tuberculosis*** dihydrolipamide succinyltransferase. MO-28, MO-31 and MO-32 were found to be identical and to show some homology to a previously identified M. ***tuberculosis*** protein. MO-33 was found to show some homology to a previously identified 14 kDa M. ***tuberculosis*** heat shock protein.

DETD . . . homologies to MO-39. MO-12, MO-13 and MO-19 were found to show some homologies to unknown sequences previously isolated from M.

tuberculosis .

DETD Isolation of Dna Sequences Encoding M. ***Tuberculosis*** Antigens by Screening of a Novel Expression Library

DETD This example illustrates isolation of DNA sequences encoding M. ***tuberculosis*** antigens by screening of a novel expression library with sera from M. ***tuberculosis*** -infected patients that were shown to be unreactive with a panel of the recombinant M. ***tuberculosis*** antigens ThRa11, ThRa3, Tb38-1, TMH4, TbF and 38 kD.

DETD Genomic DNA from M. ***tuberculosis*** Erdman strain was randomly sheared to an average size of 2 kb, and blunt ended with Klenow polymerase, followed by. . . Wis.) and packaged in vitro using the PhageMaker extract (Novagen). The resulting library was screened with sera from several M. ***tuberculosis*** donors that had been shown to be negative on a panel of previously identified M. ***tuberculosis*** antigens as described above in Example 3B.

DETD . . . 324-326, 328, 330, 332, 334 and 336 were found to show some homology to unknown sequences previously identified in M. ***tuberculosis*** .

DETD Isolation of Soluble M. ***Tuberculosis*** ANTIGENS Using Mass Spectetry

DETD This example illustrates the use of mass spectrometry to identify soluble M. ***tuberculosis*** antigens.

DETD In a first approach, M. ***tuberculosis*** culture filtrate was screened by Western analysis using serum from a ***tuberculosis*** -infected individual. The reactive bands were excised from a silver stained gel and the amino acid sequences determined by mass spectrometry.. . . of this sequence with those in the gene bank revealed homology to the 85b precursor antigen previously identified in M. ***tuberculosis*** .

DETD In a second approach, the high molecular weight region of M. ***tuberculosis*** culture supernatant was studied. This area may contain immunodominant antigens which may be useful in the diagnosis of M. ***tuberculosis*** infection. Two known monoclonal antibodies, IT42 and IT57 (available from the Center for Disease Control, Atlanta, Ga.), show reactivity by. . . the antigens remains unknown. In addition, unknown high-molecular weight proteins have been described as containing a surrogate marker for M. ***tuberculosis*** infection in HIV-positive individuals (Jnl. Infect. Dis., 176:133-143, 1997). To determine the identity of these antigens, two-dimensional gel electrophoresis and. . . phosphoenolpyruvate kinase. The two sequences isolated from spot 2 were determined to be from two DNAks, previously identified in M. ***tuberculosis*** as heat shock proteins. Spot 4 was determined to be the previously identified M. ***tuberculosis*** protein Kat G. To the best of the inventors' knowledge, neither PcK-1 nor the two DNAks have previously been shown to have utility in the diagnosis of M. ***tuberculosis*** infection.

DETD Use of Representive Antigens for Serodiagnosis of ***Tuberculosis***

DETD . . . ELISA reactivity of two recombinant antigens isolated using method A in Example 3 (ThRa3 and TbRa9) with sera from M. ***tuberculosis*** positive and negative patients. The reactivity of these antigens is compared to that of bacterial lysate isolated from M. ***tuberculosis*** strain H37Ra (Difco, Detroit, Mich.). In both cases, the recombinant antigens differentiated positive from negative sera. Based on cut-off values. . .

DETD The reactivity of four antigens (TbRa3, ThRa9, TbH4 and TbH12) with sera from a group of M. ***tuberculosis*** infected patients with differing reactivity in the acid fast stain of sputum (Smithwick and David, Tubercle 52:226, 1971) was also examined, and compared to the reactivity of M. ***tuberculosis*** lysate and the 38 kD antigen. The results are presented in Table 3, below:

DETD
TABLE 3

REACTIVITY OF ANTIGENS WITH SERA FRQM M. ***TUBERCULOSIS*** PATIENTS
ELISA Values
Patient Acid Fast Sputum Lysate 38kD TbRa9 TbH12 TbH4 TbRa3

Tb01B93I-2 ++++ 1.853 0.634 0.998 1.022 1.030 1.314
Tb01B93I-19 ++++. . .

DETD . . . sensitivity of 27 out of 27, indicating that these antigens should complement each other in the serological detection of M. ***tuberculosis*** infection. In addition, several of the recombinant antigens detected positive sera that were not detected using the 38 kD antigen,. . .

DETD The reactivity of the recombinant antigen TbRall with sera from M. ***tuberculosis*** patients shown to be negative for the 38 kD antigen, as well as with sera from PPD positive and normal. . .

DETD . . . After washing, the assay was developed with TMB substrate as described above. The reactivity of TbRa2A with sera from M. ***tuberculosis*** patients and normal donors in shown in Table 4. The mean value for reactivity of ThRa2A with sera from M. ***tuberculosis*** patients was 0.444 with a standard deviation of 0.309. The mean for reactivity with sera from normal donors was 0.109. . .

DETD
TABLE 4

REACTIVITY OF TBRA2A WITH SERA FROM
M. ***TUBERCULOSIS*** PATIENTS AND FROM
NORMAL DONORS
Serum ID Status OD 450

Tb85 TB 0.680
Tb86 TB 0.450
Tb87 TB 0.263
Tb88. . .

DETD The reactivity of the recombinant antigen (g) (SEQ ID NO: 60) with sera from M. ***tuberculosis*** patients and normal donors was determined by ELISA as described above. FIG. 8 shows the results of the titration of antigen (g) with four M. ***tuberculosis*** positive sera that were all reactive with the 38 kD antigen and with four donor sera. All four positive sera. . .

DETD The reactivity of the recombinant antigen TbH-29 (SEQ ID NO: 137) with sera from M. ***tuberculosis*** patients, PPD positive donors and normal donors was determined by indirect ELISA as described above. The results are shown in FIG. 9. TbH-29 detected 30 out of 60M. ***tuberculosis*** sera, 2 out of 8 PPD positive sera and 2 out of 27 normal sera.

DETD . . . results of ELISA tests (both direct and indirect) of the

antigen TbH-33 (SEQ ID NO: 140) with sera from M. ***tuberculosis*** patients and from normal donors and with a pool of sera from M. ***tuberculosis*** patients. The mean OD 450 was demonstrated to be higher with sera from M. ***tuberculosis*** patients than from normal donors, with the mean OD 450 being significantly higher in the indirect ELISA than in the direct ELISA. FIG. 11 is a titration curve for the reactivity of recombinant TbH-33 with sera from M. ***tuberculosis*** patients and from normal donors showing an increase in OD 450 with increasing concentration of antigen.

DETD The reactivity of the recombinant antigens RDIF6, RDIF8 and RDIF10 (SEQ ID NOS: 184-187, respectively) with sera from M. ***tuberculosis*** patients and normal donors was determined by ELISA as described above. RDIF6 detected 6 out of M. ***tuberculosis*** sera and 0 out of 15 normal sera; RDIF8 detected 14 out of 32 M. ***tuberculosis*** sera and 0 out of 15 normal sera; and RDIF10 detected 4 out of 27 M ***tuberculosis*** sera and 1 out of 15 normal sera. In addition, RDIF 10 was found to detect 0 out of 5. . .

DETD . . . 5, were expressed in E. coli and purified using a hexahistidine tag. The reactivity of these antigens with both M. ***tuberculosis*** positive and negative sera was examined by ELISA as described above. Titration curves showing the reactivity of MO-1, MO-2, MO-4, MO-28 and MO-29 at different solid phase coat levels when tested against four M. ***tuberculosis*** positive sera and four M. ***tuberculosis*** negative sera are shown in FIGS. 12A-E, respectively. Three of the clones, MO-1, MO-2 and MO-29 were further tested on panels of HIV positive/ ***tuberculosis*** (HIV/TB) positive and extrapulmonary sera. MO-1 detected 3/20 extrapulmonary and 2/38 HIV/TB sera. On the same sera groups, MO-2 detected. . . and 16/38 HIV/TB sera. In addition, MO-1 detected 6/17 sera that had previously been shown only to react with M. ***tuberculosis*** lysate and not with either 38 kD or with other antigens of the subject invention.

DETD Preparation and Characterization of M. ***Tuberculosis*** Fusion Proteins

DETD Genomic M. ***tuberculosis*** DNA was used to PCR full-length TbH4 (FL TbH4) with the primers PDM-157 and PDM-160 (SEQ ID NO: 343 and. . .

DETD Use of M. ***Tuberculosis*** Fusion Proteins For Serodiagnosis of ***Tuberculosis***

DETD The effectiveness of the fusion protein ThRa3-38 kD-Tb38-1, prepared as described above, in the serodiagnosis of ***tuberculosis*** infection was examined by ELISA.

DETD . . . 6, with the fusion protein being coated at 200 ng/well. A panel of sera was chosen from a group of ***tuberculosis*** patients previously shown, either by ELISA or by western blot analysis, to react with each of the three antigens individually. . .

DETD

TABLE 5

REACTIVITY OF TRI-PEPTIDE FUSION PROTEIN WITH SERA FROM M. ***TUBERCULOSIS*** PATIENTS

ELISA and/or Western Blot Reactivity Fusion Fusion with Individual proteins Recombinant Recombinant Serum ID Status 38kd Tb38-1 TbRa3 OD 450. . .

DETD The reactivity of the fusion protein TbF-2 with sera from M. ***tuberculosis*** -infected patients was examined by ELISA using the

protocol described above. The results of these studies (Table 6) demonstrate that all. . .

DETD . . . Tyr
100

SEQUENCE CHARACTERISTICS:
LENGTH: 53 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 141
GGATCCATAT GGGCCATCAT CATCATCATC ACGTGATCGA CATCATCGGG ACC 53

SEQUENCE CHARACTERISTICS:
LENGTH: 42 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR Primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 142
CCTGAATTCA GGCCTCGGTT GCGCCGGCCT CATCTTGAAC GA 42

SEQUENCE CHARACTERISTICS:
LENGTH: 31 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR Primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 143
GGATCCTGCA GGCTCGAAAC CACCGAGCGG T 31

SEQUENCE CHARACTERISTICS:
LENGTH: 31 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 144
CTCTGAATTC AGCGCTGGAA ATCGTCGCGA T 31

SEQUENCE CHARACTERISTICS:
LENGTH: 33 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"

ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 145
GGATCCAGCG CTGAGATGAA GACCGATGCC GCT 33
SEQUENCE CHARACTERISTICS:
LENGTH: 33 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 146
GAGAGAATTC TCAGAAGCCC ATTTGCGAGG ACA 33
SEQUENCE CHARACTERISTICS:
LENGTH: 1993 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: DNA (genomic)
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
FEATURE:
NAME/KEY: CDS
LOCATION: 152..1273
SEQUENCE: 147
TGTTCTTCGA CGGCAGGCTG GTGGAGGAAG GGCCCACCGA ACAGCTGTTC TCCTCGCCGA 60
AGCATGCGGA AACCGCCCGA TACGTCGCCG GACTGTCGGG GGACGTCAAG GACGCCAAGC 120
GCGGAAATTG AAGAGCACAG AAAGGTATGG C GTG AAA. . .
CLM What is claimed is:
1. A method for detecting M. ***tuberculosis*** infection in a biological sample, the method comprising: (a) contacting the biological sample with a polypeptide comprising an amino acid. . . NO: 346; and (b) detecting in the sample the presence of antibodies that bind to the poleptide, thereby detecting M. ***tuberculosis*** infection in the biological sample.

L15 ANSWER 5 OF 7 USPATFULL on STN
AN 2002:39663 USPATFULL
TI Compositions and methods for the prevention and treatment of M. ***tuberculosis*** infection
IN Reed, Steven G., Bellevue, WA, United States
Skeiky, Yasir A. W., Seattle, WA, United States
Dillon, Davin C., Redmond, WA, United States
PA Corixa Corporation, Seattle, WA, United States (U.S. corporation)
PI US 6350456 B1 20020226
AI US 1998-56556 19980407 (9)
RLI Continuation-in-part of Ser. No. US 1998-25197, filed on 18 Feb 1998, now abandoned Continuation-in-part of Ser. No. US 1997-942578, filed on 1 Oct 1997, now abandoned Continuation-in-part of Ser. No. US 1997-818112, filed on 13 Mar 1997
DT Utility
FS GRANTED

EXNAM Primary Examiner: Swartz, Rodney P
LREP Townsend and Townsend and Crew LLP
CLMN Number of Claims: 10
ECL Exemplary Claim: 1
DRWN 23 Drawing Figure(s); 14 Drawing Page(s)
LN.CNT 6417
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compositions and methods for treatment and vaccination against ***tuberculosis*** are disclosed. In one aspect the compositions provided include at least two polypeptides that contain an immunogenic portion of a M. ***tuberculosis*** antigen or at least two DNA molecules encoding such polypeptides. In a second aspect, the compositions provided include a fusion protein comprising at least two polypeptides that contain an immunogenic portion of a M. ***tuberculosis*** antigen. Such compositions may be formulated into vaccines and/or pharmaceutical compositions for immunization against M. ***tuberculosis*** infection, or may be used for the diagnosis of ***tuberculosis*** .

TI Compositions and methods for the prevention and treatment of M. ***tuberculosis*** infection

AB Compositions and methods for treatment and vaccination against ***tuberculosis*** are disclosed. In one aspect the compositions provided include at least two polypeptides that contain an immunogenic portion of a M. ***tuberculosis*** antigen or at least two DNA molecules encoding such polypeptides. In a second aspect, the compositions provided include a fusion protein comprising at least two polypeptides that contain an immunogenic portion of a M. ***tuberculosis*** antigen. Such compositions may be formulated into vaccines and/or pharmaceutical compositions for immunization against M. ***tuberculosis*** infection, or may be used for the diagnosis of ***tuberculosis*** .

SUMM The present invention relates generally to compositions for the prevention and treatment of ***tuberculosis*** . The invention is more particularly related to compositions comprising at least two Mycobacterium ***tuberculosis*** antigens, and the use of such compositions for treating and vaccinating against Mycobacterium ***tuberculosis*** infection.

SUMM ***Tuberculosis*** is a chronic, infectious disease, that is generally caused by infection with Mycobacterium ***tuberculosis*** . It is a major disease in developing countries, as well as an increasing problem in developed areas of the world,. . .

SUMM Although ***tuberculosis*** can generally be controlled using extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease.. . .

SUMM Inhibiting the spread of ***tuberculosis*** requires effective vaccination and accurate, early diagnosis of the disease. Currently, vaccination with live bacteria is the most efficient method. . .

SUMM While macrophages have been shown to act as the principal effectors of M. ***tuberculosis*** immunity, T cells are the predominant inducers of such immunity. The essential role of T cells in protection against M. ***tuberculosis*** infection is illustrated by the frequent occurrence of M. ***tuberculosis*** in AIDS patients, due to the depletion of CD4+ T cells associated with human immunodeficiency virus (HIV) infection. Mycobacterium-reactive CD4+. . . that 1,25-dihydroxy-vitamin D3, either alone or in combination with IFN-.gamma. or tumor

necrosis factor-alpha, activates human macrophages to inhibit M. ***tuberculosis*** infection. Furthermore, it is known that IFN-.gamma. stimulates human macrophages to make 1,25-dihydroxy-vitamin D3. Similarly, IL-12 has been shown to play a role in stimulating resistance to M. ***tuberculosis*** infection. For a review of the immunology of M. ***tuberculosis*** infection see Chan and Kaufmann in ***Tuberculosis*** : Pathogenesis, Protection and Control, Bloom (ed.), ASM Press, Washington, D.C., 1994.

SUMM Accordingly, there is a need in the art for improved compositions and methods for preventing and treating ***tuberculosis*** .

SUMM Briefly stated, this invention provides compositions and methods for preventing and treating M. ***tuberculosis*** infection. In one aspect, pharmaceutical compositions are provided that comprise a physiologically acceptable carrier and either (a) a first polypeptide. . . including a first polypeptide and a second polypeptide, wherein each of the polypeptides comprises an immunogenic portion of a M. ***tuberculosis*** antigen or a variant thereof. In specific embodiments, the first polypeptide comprises an immunogenic portion of a M. ***tuberculosis*** antigen having an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO: 91, 107, 109, 111 and variants thereof, and the second polypeptide comprises an immunogenic portion of a M. ***tuberculosis*** antigen having an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO: 79, 88,. . .

SUMM . . . DNA molecule and a second DNA molecule, wherein each of the DNA molecules encodes an immunogenic portion of a M. ***tuberculosis*** antigen or a variant thereof. In specific embodiments, the first DNA molecule comprises a nucleotide sequence selected from the group. . .

SUMM . . . including a first polypeptide and a second polypeptide, wherein each of the polypeptides comprises an immunogenic portion of a M. ***tuberculosis*** antigen or a variant thereof. In specific embodiments, the first polypeptide comprises an immunogenic portion of a M. ***tuberculosis*** antigen having an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO: 91, 107, 109, 111 and variants thereof, and the second polypeptide comprises an immunogenic portion of a M. ***tuberculosis*** antigen having an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO: 79, 88,. . .

SUMM . . . DNA molecule and a second DNA molecule, wherein each of the DNA molecules encodes an immunogenic portion of a M. ***tuberculosis*** antigen or a variant thereof. In specific embodiments, the first DNA molecule comprises a nucleotide sequence selected from the group. . .

SUMM . . . B illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a first and a second M. ***tuberculosis*** -immune donor, respectively, by the 14 Kd, 20 Kd and 26 Kd antigens described in Example 1.

SUMM FIG. 2 illustrates the stimulation of proliferation and interferon-y production in T cells derived from an M. ***tuberculosis*** -immune individual by the two representative polypeptides TbRa3 and TbRa9.

SUMM FIGS. 3A-D illustrate the reactivity of antisera raised against secretory M. ***tuberculosis*** proteins, the known M. ***tuberculosis*** antigen 85b and the inventive antigens Tb38-1 and TbH-9, respectively, with M. ***tuberculosis*** lysate (lane 2), M. ***tuberculosis*** secretory proteins (lane 3), recombinant Tb38-1 (lane 4), recombinant TbH-9 (lane 5) and recombinant 85b (lane 5).

SUMM FIG. 4A illustrates the stimulation of proliferation in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, recombinant TbH-9 and a control antigen, TbRa11.

SUMM FIG. 4B illustrates the stimulation of interferon-.gamma. production in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, PPD and recombinant TbH-9.

SUMM FIGS. 8A and B illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a first M. ***tuberculosis*** -immune individual by the representative polypeptides XP-1, RDIF6, RDIF8, RDIF10 and RDIF11.

SUMM FIGS. 9A and B illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a second M. ***tuberculosis*** -immune individual by the representative polypeptides XP-1, RDIF6, RDIF8, RDIF10 and RDIF11.

SUMM FIG. 10 illustrates the percentage survival of monkeys infected with M. ***tuberculosis*** following immunization with either saline, AS2 adjuvant alone, recombinant TbH9 (referred to as Mtb39) formulated in AS2 adjuvant, recombinant TbH9. . .

SUMM FIGS. 11A and B illustrate the bacteriological burden in the lungs and spleens, respectively, of guinea pigs infected with M. ***tuberculosis*** following immunization with either recombinant TbH9 (referred to as Mtb39) alone, recombinant TbRa35 (referred to as Mtb32) alone, or a. . .

SUMM FIG. 12 illustrates the bacteriological burden in the lungs of mice challenged with M. ***tuberculosis*** following immunization with either TbRa1 DNA alone, TbH9 DNA plus TbRa35 DNA, or a combination of TbH9 DNA, TbRa35 DNA. . .

SUMM SEQ ID NO: 154 is the DNA sequence of the M. ***tuberculosis*** antigen 38 kD.

SUMM SEQ ID NO: 155 is the amino acid sequence of the M. ***tuberculosis*** antigen 38 kD.

SUMM As noted above, the present invention is generally directed to compositions and methods for the prevention and treatment of ***tuberculosis*** . In one aspect, the compositions of the subject invention include at least two isolated polypeptides that comprise an immunogenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen. The inventive compositions may comprise a mixture of at least two isolated. . .

SUMM . . . or, alternatively, at least two DNA molecules encoding such polypeptides, are particularly efficacious in the induction of protective immunity against ***tuberculosis*** . In preferred embodiments, the inventive compositions comprise at least an immunogenic portion of the M. ***tuberculosis*** antigen TbH9, together with at least an immunogenic portion of either the M. ***tuberculosis*** antigen TbRa35 or the M. ***tuberculosis*** antigen Tb38.1. However, other combinations of the M. ***tuberculosis*** antigens described herein may also be effectively used in the treatment and prevention of ***tuberculosis*** , as may combinations of the inventive antigens with known M. ***tuberculosis*** antigens, such as the previously described 38 kD antigen (Andersen and Hansen, Infect. Immun. 57:2481-2488, 1989; Genbank Accession No. M30046).

SUMM . . . the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising an immunogenic portion of a M. ***tuberculosis*** antigen may consist entirely of the immunogenic portion, or may contain additional sequences. The additional sequences

may be derived from the native M. ***tuberculosis*** antigen or may be heterologous, and such sequences may (but need not) be immunogenic. In general, the polypeptides disclosed herein. . .

SUMM . . . from the group of T cells, NK cells, B cells and macrophages, where the cells are derived from an M. ***tuberculosis*** -immune individual. Immunogenic portions of the antigens described herein may be prepared and identified using well known techniques, such as those. .
.

SUMM . . . modifications, such that the therapeutic, antigenic and/or immunogenic properties of the polypeptide are retained. A variant of a specific M. ***tuberculosis*** antigen will therefore stimulate cell proliferation and/or IFN-gamma in Th1 cells raised against that specific antigen. Polypeptide variants preferably exhibit. . .

SUMM In general, M. ***tuberculosis*** antigens, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures, as described in. . .

SUMM . . . antigen, which has been inserted into an expression vector and expressed in an appropriate host cell. DNA sequences encoding M. ***tuberculosis*** antigens may, for example, be identified by screening an appropriate M. ***tuberculosis*** genomic or cDNA expression library with sera obtained from patients infected with M. ***tuberculosis*** . Such screens may generally be performed using techniques well known to those of ordinary skill in the art, such as. .
.

SUMM DNA sequences encoding M. ***tuberculosis*** antigens may also be obtained by screening an appropriate M. ***tuberculosis*** cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of. . .

SUMM Alternatively, genomic or cDNA libraries derived from M. ***tuberculosis*** may be screened directly using peripheral blood mononuclear cells (PBMCs) or T cell lines or clones derived from one or more M. ***tuberculosis*** -immune individuals. In general, PBMCs and/or T cells for use in such screens may be prepared as described below. Direct library. . . of expressed recombinant proteins for the ability to induce proliferation and/or interferon-.gamma. production in T cells derived from an M. ***tuberculosis*** -immune individual.

SUMM . . . cytokine production (i.e., interferon-.gamma. and/or interleukin-12 production) in T cells, NK cells, B cells and/or macrophages derived from an M. ***tuberculosis*** -immune individual. The selection of cell type for use in evaluating an immunogenic response to a antigen will, of course, depend. . . the desired response. For example, interleukin-12 production is most readily evaluated using preparations containing B cells and/or macrophages. An M. ***tuberculosis*** -immune individual is one who is considered to be resistant to the development of ***tuberculosis*** by virtue of having mounted an effective T cell response to M. ***tuberculosis*** (i.e., substantially free of disease symptoms). Such individuals may be identified based on a strongly positive (i.e., greater than about 10 mm diameter induration) intradermal skin test response to ***tuberculosis*** proteins (PPD) and an absence of any signs or symptoms of ***tuberculosis*** disease. T cells, NK cells, B cells and macrophages derived from M. ***tuberculosis*** -immune individuals may be prepared using methods known to those of ordinary skill in the art. For example, a preparation of. . . individual mycobacterial proteins, may be employed. Such T cell clones may be

generated by, for example, culturing PBMCs from M. ***tuberculosis*** -immune individuals with mycobacterial proteins for a period of 2-4 weeks. This allows expansion of only the mycobacterial protein-specific T cells,. . . production (i.e., interferon-.gamma. and/or interleukin-12 production) performed using T cells, NK cells, B cells and/or macrophages derived from an M. ***tuberculosis*** -immune individual are considered immunogenic. Such assays may be performed, for example, using the representative procedures described below. Immunogenic portions of. . .

SUMM . . . and/or interleukin-12 production) in T cells, NK cells, B cells and/or macrophages derived from at least about 25% of M. ***tuberculosis*** -immune individuals. Among these immunogenic antigens, polypeptides having superior therapeutic properties may be distinguished based on the magnitude of the responses. . . proliferation and/or cytokine production in vitro in cells derived from more than about 25% of individuals who are not M. ***tuberculosis*** -immune, thereby eliminating responses that are not specifically due to M. ***tuberculosis*** -responsive cells. Those antigens that induce a response in a high percentage of T cell, NK cell, B cell and/or macrophage preparations from M. ***tuberculosis*** -immune individuals (with a low incidence of responses in cell preparations from other individuals) have superior therapeutic properties.

SUMM Antigens with superior therapeutic properties may also be identified based on their ability to diminish the severity of M. ***tuberculosis*** infection in experimental animals, when administered as a vaccine. Suitable vaccine preparations for use on experimental animals are described in. . .

SUMM Portions and other variants of M. ***tuberculosis*** antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally. . .

SUMM . . . comprises a first and a second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known M. ***tuberculosis*** antigen, such as the 38 kD antigen discussed above, together with variants of such fusion proteins. The fusion proteins of. . . present invention comprise a first and a second isolated DNA molecule, each isolated DNA molecule encoding either an inventive M. ***tuberculosis*** antigen or a known M. ***tuberculosis*** antigen.

SUMM . . . present invention are preferably formulated as either pharmaceutical compositions or as vaccines for in the induction of protective immunity against ***tuberculosis*** in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be. . . may be free of detectable disease and/or infection. In other words, protective immunity may be induced to prevent or treat ***tuberculosis*** .

SUMM . . . above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known M. ***tuberculosis*** antigen, such as the 38 kD antigen described above. For example, administration of DNA encoding a polypeptide of the present. . .

SUMM . . . aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune responses, such as lipid A, Bortadella pertussis or Mycobacterium ***tuberculosis*** . Suitable adjuvants are commercially available and include, for example, Freund's Incomplete Adjuvant and Freund's Complete Adjuvant (Difco Laboratories) and Merck.

. .

SUMM . . . described above, is capable of raising an immune response in an immunized patient sufficient to protect the patient from M. ***tuberculosis*** infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced in situ. . .

DETD PURIFICATION AND CHARACTERIZATION OF POLYPEPTIDES FROM M. ***TUBERCULOSIS*** CULTURE FILTRATE

DETD This example illustrates the preparation of M. ***tuberculosis*** soluble polypeptides from culture filtrate. Unless otherwise noted, all percentages in the following example are weight per volume.

DETD M. ***tuberculosis*** (either H37Ra, ATCC No. 25177, or H37Rv, ATCC No. 25618) was cultured in sterile GAS media at 37.degree. C. for. . .

DETD Additional soluble antigens were isolated from M. ***tuberculosis*** culture filtrate as follows. M. ***tuberculosis*** culture filtrate was prepared as described above. Following dialysis against Bis-Tris propane buffer, at pH 5.5, fractionation was performed using. . .

DETD . . . sequences that encode the antigens designated as (a), (c), (d) and (g) above were obtained by screening a genomic M. ***tuberculosis*** library using .sup.32P end labeled degenerate oligonucleotides corresponding to the N-terminal sequence and containing M. ***tuberculosis*** codon bias. The screen performed using a probe corresponding to antigen (a) above identified a clone having the sequence provided. . .

DETD . . . was amplified from genomic DNA using the sequence obtained from GENBANK. This sequence was then used to screen the M. ***tuberculosis*** library described below in Example 2 and a full length copy of the M. ***tuberculosis*** homologue was obtained (SEQ ID No. 99).

DETD The amino acid sequence for antigen (j) was found to be homologous to a known M. ***tuberculosis*** protein translated from a DNA sequence. To the best of the inventors' knowledge, this protein has not been previously shown. . .

DETD USE OF PATIENT SERA TO ISOLATE M. ***TUBERCULOSIS*** ANTIGENS

DETD This example illustrates the isolation of antigens from M. ***tuberculosis*** lysate by screening with serum from M. ***tuberculosis*** -infected individuals.

DETD Dessicated M. ***tuberculosis*** H37Ra (Difco Laboratories) was added to a 2% NP40 solution, and alternately homogenized and sonicated three times. The resulting suspension. . . Centriprep 10 (Amicon, Beverley, Mass.) and then screened by Western blot for serological activity using a serum pool from M. ***tuberculosis*** -infected patients which was not immunoreactive with other antigens of the present invention.

DETD A DNA sequence that encodes the antigen designated as (m) above was obtained by screening a genomic M. ***tuberculosis*** Erdman strain library using labeled degenerate oligonucleotides corresponding to the N-terminal sequence of SEQ ID NO: 137. A clone was. . . 204. Comparison of these sequences with those in the gene bank revealed some similarity to sequences previously identified in M. ***tuberculosis*** and M. bovis.

DETD PREPARATION OF DNA SEQUENCES ENCODING M. ***TUBERCULOSIS*** ANTIGENS

DETD This example illustrates the preparation of DNA sequences encoding M. ***tuberculosis*** antigens by screening a M. ***tuberculosis*** expression library with sera obtained from patients infected with M. ***tuberculosis*** , or with anti-sera raised against soluble M.

tuberculosis antigens.

DETD A. PREPARATION OF M. ***TUBERCULOSIS*** SOLUBLE ANTIGENS USING RABBIT ANTI-SERA RAISED AGAINST M. ***TUBERCULOSIS*** SUPERNATANT

DETD Genomic DNA was isolated from the M. ***tuberculosis*** strain H37Ra. The DNA was randomly sheared and used to construct an expression library using the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Rabbit anti-sera was generated against secretory proteins of the M. ***tuberculosis*** strains H37Ra, H37Rv and Erdman by immunizing a rabbit with concentrated supernatant of the M. ***tuberculosis*** cultures. Specifically, the rabbit was first immunized subcutaneously with 200 .mu.g of protein antigen in a total volume of 2. . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD Thirty two clones were purified. Of these, 25 represent sequences that have not been previously identified in human M. ***tuberculosis*** . Recombinant antigens were expressed and purified antigens used in the immunological analysis described in Example 1. Proteins were induced by. . .

DETD . . . ID Nos. 76, 68, 70, 75) show some homology to sequences previously identified in Mycobacterium leprae but not in M. ***tuberculosis*** . TbRA2A was found to be a lipoprotein, with a six residue lipidation sequence being located adjacent to a hydrophobic secretory sequence. TbRA11, TbRA26, TbRA28 and TbDPEP (SEQ ID Nos.: 65, 73, 74, 53) have been previously identified in M. ***tuberculosis*** . No significant homologies were found to TbRA1, TbRA3, TbRA4, TbRA9, TbRA10, TbRA13, TbRA17, TbRa19, TbRA29, TbRA32, TbRA36 and the overlapping. . .

DETD . . . results of PBMC proliferation and interferon-.gamma. assays performed on representative recombinant antigens, and using T-cell preparations from several different M. ***tuberculosis*** -immune patients, are presented in Tables 2 and 3, respectively.

DETD These results indicate that these soluble antigens can induce proliferation and/or interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** -immune individual.

DETD B. USE OF SERA FROM PATIENTS HAVING PULMONARY OR PLEURAL ***TUBERCULOSIS*** TO IDENTIFY DNA SEQUENCES ENCODING M. ***TUBERCULOSIS*** ANTIGENS

DETD . . . DNA library described above, and an additional H37Rv library, were screened using pools of sera obtained from patients with active ***tuberculosis*** . To prepare the H37Rv library, M. ***tuberculosis*** strain H37Rv genomic DNA was isolated, subjected to partial Sau3A digestion and used to construct an expression library using the. . . and TbH=high reactivity) in both ELISA and immunoblot format. A fourth pool of sera from seven patients with active pulmonary ***tuberculosis*** was also employed. All of the sera lacked increased reactivity with the recombinant 38 kD M. ***tuberculosis*** H37Ra phosphate-binding protein.

DETD . . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD Thirty two clones were purified. Of these, 31 represented sequences that had not been previously identified in human M. ***tuberculosis*** . Representative sequences of the DNA molecules identified are provided in SEQ ID Nos.: 26-51 and 105. Of these, TbH-8-2 (

DETD . . . of the open reading frame for the antigen ESAT-6 previously identified in M. bovis (Acc. No. U34848) and in M. ***tuberculosis*** (Sorensen et al., Infec. Immun. 63:1710-1717, 1995).

DETD . . . sequence of Tb38-1F3 is presented in SEQ. ID. NO. 117. A TbH-9 probe identified three clones in the H37Rv library: ***TbH*** - ***9*** - ***FL*** (SEQ. ID NO. 106), which may be the homologue of TbH-9 (R37Ra), TbH-9-1 (SEQ. ID NO. 108), and TbH-9-4 (SEQ.. . .

DETD Further screening of the M. ***tuberculosis*** genomic DNA library, as described above, resulted in the recovery of ten additional reactive clones, representing seven different genes. One. . . determined to be identical to the 14 Kd alpha crystallin heat shock protein previously shown to be present in M. ***tuberculosis*** , and a third was determined to be identical to the antigen TbH-8 described above. The determined DNA sequences for the. . . contains the reactive open reading frame), although the 3' end of TbH-29 was found to be identical to the M. ***tuberculosis*** cosmid Y227. TbH-32 and TbH-33 were found to be identical to the previously identified M. ***tuberculosis*** insertion element IS6110 and to the M. ***tuberculosis*** cosmid Y50, respectively. No significant homologies to TbH-30 were found.

DETD . . . and uninduced lysates were run in duplicate on SDS-PAGE and transferred to nitrocellulose filters. Filters were reacted with human M. ***tuberculosis*** sera (1:200 dilution) reactive with TbH and a rabbit sera (1:200 or 1:250 dilution) reactive with the N-terminal 4 Kd. . .

DETD Positive reaction of the recombinant human M. ***tuberculosis*** antigens with both the human M. ***tuberculosis*** sera and anti-lacZ sera indicate that reactivity of the human M. ***tuberculosis*** sera is directed towards the fusion protein. Antigens reactive with the anti-lacZ sera but not with the human M. ***tuberculosis*** sera may be the result of the human M. ***tuberculosis*** sera recognizing conformational epitopes, or the antigen-antibody binding kinetics may be such that the 2 hour sera exposure in the. . .

DETD These results indicate that both the inventive M. ***tuberculosis*** antigens and ESAT-6 can induce proliferation and/or interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** -immune individual. To the best of the inventors' knowledge, ESAT-6 has not been previously shown to stimulate human immune responses.

DETD . . . help to localize T-cell epitopes within Tb38-1 capable of inducing proliferation and interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** immune individual.

DETD Studies were undertaken to determine whether the antigens TbH-9 and Tb38-1 represent cellular proteins or are secreted into M. ***tuberculosis*** culture media. In the first study, rabbit sera were raised against A) secretory proteins of M. ***tuberculosis*** , B) the known secretory recombinant M. ***tuberculosis*** antigen 85b, C) recombinant Tb38-1 and D) recombinant TbH-9, using protocols substantially the same as that as described in Example 3A. Total M. ***tuberculosis*** lysate, concentrated supernatant of M. ***tuberculosis*** cultures and the recombinant antigens 85b, TbH-9 and Tb38-1 were resolved on denaturing gels, immobilized on nitrocellulose membranes and duplicate. . .

DETD . . . FIGS. 3A-D, respectively, wherein the lane designations are as follows: 1) molecular weight protein standards; 2) 5 .mu.g of M.

tuberculosis lysate; 3) 5 .mu.g secretory proteins; 4) 50 ng recombinant Tb38-1; 5) 50 ng recombinant TbH-9; and 6) 50 ng. . . by an arrow). These results demonstrate that Tb38-1 and TbH-9 are intracellular antigens and are not actively secreted by M. ***tuberculosis*** .

DETD . . . an intracellular antigen was confirmed by determining the reactivity of TbH-9-specific human T cell clones to recombinant TbH-9, secretory M. ***tuberculosis*** proteins and PPD. A TbH-9-specific T cell clone (designated 131TbH-9) was generated from PBMC of a healthy PPD-positive donor. The proliferative response of 131TbH-9 to secretory proteins, recombinant TbH-9 and a control M. ***tuberculosis*** antigen, TbRa11, was determined by measuring uptake of tritiated thymidine, as described in Example 1. As shown in FIG. 4A, the clone 131TbH-9 responds specifically to TbH-9, showing that TbH-9 is not a significant component of M. ***tuberculosis*** secretory proteins. FIG. 4B shows the production of IFN-.gamma. by a second TbH-9-specific T cell clone (designated PPD 800-10) prepared. . . cell clone with secretory proteins, PPD or recombinant TbH-9. These results further confirm that TbH-9 is not secreted by M. ***tuberculosis*** .

DETD C. USE OF SERA FROM PATIENTS HAVING EXTRAPULMONARY ***TUBERCULOSIS*** TO IDENTIFY DNA SEQUENCES ENCODING M. ***TUBERCULOSIS*** ANTIGENS

DETD Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). The resulting library was screened using pools of sera obtained from individuals with extrapulmonary ***tuberculosis*** , as described above in Example 3B, with the secondary antibody being goat anti-human IgG+A+M (H+L) conjugated with alkaline phosphatase.

DETD . . . the exception of the 3' ends of XP2 and XP6 which were found to bear some homology to known M. ***tuberculosis*** cosmids. The DNA sequences for XP27 and XP36 are shown in SEQ ID Nos.: 163 and 164, respectively, with the. . .

DETD . . . described herein, recombinant XP1 was found to stimulate cell proliferation and IFN-.gamma. production in T cells isolated from an M. ***tuberculosis*** -immune donors.

DETD D. PREPARATION OF M. ***TUBERCULOSIS*** SOLUBLE ANTIGENS USING RABBIT ANTI-SERA RAISED AGAINST M. ***TUBERCULOSIS*** FRACTIONATED PROTEINS

DETD M. ***tuberculosis*** lysate was prepared as described above in Example 2. The resulting material was fractionated by HPLC and the fractions screened by Western blot for serological activity with a serum pool from M. ***tuberculosis*** -infected patients which showed little or no immunoreactivity with other antigens of the present invention. Rabbit anti-sera was generated against the most reactive fraction using the method described in Example 3A. The anti-sera was used to screen an M. ***tuberculosis*** Erdman strain genomic DNA expression library prepared as described above. Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones determined.

DETD . . . Of these, one was found to be TbRa35, described above, and one was found to be the previously identified M. ***tuberculosis*** antigen, HSP60. Of the remaining eight clones, seven (hereinafter referred to as RDIF2, RDIF5, RDIF8, RDIF10, RDIF11 and RDIF12) were found to bear some similarity to previously identified M.

tuberculosis sequences. The determined DNA sequences for RDIF2, RDIF5, RDIF8, RDIF10 and RDIF11 are provided in SEQ ID Nos.: 189-193, respectively,. . .

DETD . . . 8A-B and 9A-B, these antigens were found to stimulate cell proliferation and IFN-.gamma. production in T cells isolated from M. ***tuberculosis*** -immune donors.

DETD An M. ***tuberculosis*** polypeptide was isolated from tuberculin purified protein derivative (PPD) as follows.

DETD . . . F. et al., "Tuberculin purified protein derivative. Preparation and analysis of a large quantity for standardm" The American Review of ***Tuberculosis*** 44:9-25, 1941).

DETD M. ***tuberculosis*** Rv strain was grown for 6 weeks in synthetic medium in roller bottles at 37.degree. C. Bottles containing the bacterial. . .

DETD Six fractions were collected, dried, suspended in PBS and tested individually in M. ***tuberculosis*** -infected guinea pigs for induction of delayed type hypersensitivity (DTH) reaction. One fraction was found to induce a strong DTH reaction. . . 80 .mu.l/minute. Eluent was monitored at 215 nm. Eight fractions were collected and tested for induction of DTH in M. ***tuberculosis*** -infected guinea pigs. One fraction was found to induce strong DTH of about 16 mm induration. The other fractions did not. . .

DETD . . . were isolated and found to have the sequences shown in SEQ ID Nos.: 130-133. A subsequent search of the M. ***tuberculosis*** genome database released by the Institute for Genomic Research revealed a match of the DPPD partial amino acid sequence with a sequence present within the M. ***tuberculosis*** cosmid MTY21C12. An open reading frame of 336 bp was identified. The full-length DNA sequence for DPPD is provided in. . .

DETD USE OF SERA FROM ***TUBERCULOSIS*** -INFECTED MONKEYS TO IDENTIFY DNA SEQUENCES ENCODING M. ***TUBERCULOSIS*** ANTIGENS

DETD Genomic DNA was isolated from M. ***tuberculosis*** Erdman strain, randomly sheared and used to construct an expression library employing the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Serum samples were obtained from a cynomolgous monkey 18, 33, 51 and 56 days following infection with M. ***tuberculosis*** Erdman strain. These samples were pooled and used to screen the M. ***tuberculosis*** genomic DNA expression library using the procedure described above in Example 3C.

DETD . . . MO-35 were found to show a high degree of relatedness and showed some homology to a previously identified unknown M. ***tuberculosis*** sequence and to cosmid MTCI237. MO-2 was found to show some homology to aspartokinase from M. ***tuberculosis*** . Clones MO-3, MO-7 and MO-27 were found to be identical and to show a high degree of relatedness to MO-5. All four of these clones showed some homology to M. ***tuberculosis*** heat shock protein 70. MO-27 was found to show some homology to M. ***tuberculosis*** cosmid MTCY339. MO-4 and MO-34 were found to show some homology to cosmid SCY21B4 and M. smegmatis integration host factor, and were both found to show some homology to a previously identified, unknown M. ***tuberculosis*** sequence. MO-6 was found to show some homology to M. ***tuberculosis*** heat shock protein 65. MO-8, MO-9, MO-10, MO-26 and MO-29 were found to be highly related to each other and to show some homology to M. ***tuberculosis*** dihydrolipamide succinyltransferase. MO-28, MO-31 and MO-32 were found to be identical

and to show some homology to a previously identified M. ***tuberculosis*** protein. MO-33 was found to show some homology to a previously identified 14 kDa M. ***tuberculosis*** heat shock protein.

DETD PREPARATION AND CHARACTERIZATION OF M. ***TUBERCULOSIS*** FUSION PROTEINS

DETD The reactivity of the fusion protein TbF-2 with sera from M. ***tuberculosis*** -infected patients was examined by ELISA using the protocol described above. The results of these studies (Table 10) demonstrate that all. . .

DETD USE OF REPRESENTATIVE RECOMBINANT ANTIGENS FOR VACCINATION AGAINST M. ***TUBERCULOSIS*** INFECTION

DETD This example illustrates the effectiveness of the recombinant M. ***tuberculosis*** antigens of the present invention in inducing protective immunization against infection with M. ***tuberculosis***
.

DETD . . . BCG were used as controls. Four weeks following the last immunization, the monkeys were challenged with 10.sup.3 units viable M. ***tuberculosis*** Erdman strain by the aerosol route. Lung pathology and survival were evaluated at regular intervals thereafter.

DETD The effectiveness of recombinant antigens of the present invention in the vaccination of guinea pigs against ***tuberculosis*** was determined as follows. Hartley guinea pigs were immunized i.m. with 20 .mu.g of either recombinant TbH9 alone or a. . . viable bacteria. Four weeks after the third immunization, the guinea pigs were challenged by aerosol inhalation of 10-20 viable M. ***tuberculosis*** strain Rv. The animals were sacrificed four weeks later and the bacteriological burden in the lungs and spleens (CFU) of. . .

DETD USE OF DNA MOLECULES ENCODING M. ***TUBERCULOSIS*** ANTIGENS FOR VACCINATION AGAINST M. ***TUBERCULOSIS*** INFECTION

DETD . . . illustrate the use of DNA molecules encoding the representative antigens TbH9, TbRa35 and TbRa1 in inducing protective immunity against M. ***tuberculosis*** infection.

DETD . . . used as a positive control. Four weeks after the last immunization, mice were challenged by aerosol with 10 viable M. ***tuberculosis*** . The animals were sacrificed 30 days later and the bacteriological burden in the lungs of individual animals was determined as. . .

DETD . . . Tyr
100

SEQUENCE CHARACTERISTICS:
LENGTH: 53 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 146
GGATCCATAT GGGCCATCAT CATCATCATC ACGTGATCGA CATCATCGGG ACC 53

SEQUENCE CHARACTERISTICS:
LENGTH: 42 base pairs
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ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 147
CCTGAATTCA GGCCTCGGTT GCGCCGGCCT CATCTTGAAC GA 42
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LENGTH: 31 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
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DESCRIPTION: /desc = "PCR Primer"
ORIGINAL SOURCE:
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SEQUENCE: 148
GGATCCTGCA GGCTCGAAAC CACCGAGCGG T 31
SEQUENCE CHARACTERISTICS:
LENGTH: 31 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 149
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SEQUENCE CHARACTERISTICS:
LENGTH: 33 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 150
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SEQUENCE CHARACTERISTICS:
LENGTH: 33 base pairs
TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid
DESCRIPTION: /desc = "PCR primer"
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
SEQUENCE: 151
GAGAGAATTC TCAGAAGCCC ATTTGCGAGG ACA 33
SEQUENCE CHARACTERISTICS:
LENGTH: 1993 base pairs
TYPE: nucleic acid
STRANDEDNESS: single

TOPOLOGY: linear
MOLECULE TYPE: DNA (genomic)
ORIGINAL SOURCE:
ORGANISM: Mycobacterium ***tuberculosis***
FEATURE:
NAME/KEY: CDS
LOCATION: 152..1273
SEQUENCE: 152
TGTTCTTCGA CGGCAGGCTG GTGGAGGAAG GGCCCACCGA ACAGCTGTTC TCCTCGCCGA 60
AGCATGCGGA AACCGCCCGA TACGTCGCCG GACTGTCGGG GGACGTCAAG GACGCCAAGC 120
GCGGAAATTG AAGAGCACAG AAAGGTATGG C GTG AAA. . .
CLM What is claimed is:
. . . a purified second polypeptide, and a physiologically acceptable carrier, wherein the first polypeptide comprises an immunogenic portion of a M. ***tuberculosis*** antigen having an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO:91, 107, 109, 111, and variants thereof, and the second polypeptide comprises an immunogenic portion of a M. ***tuberculosis*** antigen having an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO:79 and variants. . .
. . . protein comprising a first polypeptide and a second polypeptide, wherein the first polypeptide comprises an immunogenic portion of a M. ***tuberculosis*** antigen having an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO:91, 107, 109, 111, and variants thereof, and the second polypeptide comprises an immunogenic portion of a M. ***tuberculosis*** antigen having an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO:79 and variants. . .

L15 ANSWER 6 OF 7 USPATFULL on STN
AN 2002:9651 USPATFULL
TI Compounds and methods for diagnosis of ***tuberculosis***
IN Reed, Steven G., Bellevue, WA, United States
Skeiky, Yasir A. W., Seattle, WA, United States
Dillon, Davin C., Redmond, WA, United States
Campos-Neto, Antonio, Bainbridge Island, WA, United States
Houghton, Raymond, Bothell, WA, United States
Vedvick, Thomas S., Federal Way, WA, United States
Twardzik, Daniel R., Bainbridge Island, WA, United States
PA Corixa Corporation, Seattle, WA, United States (U.S. corporation)
PI US 6338852 B1 20020115
AI US 1997-818111 19970313 (8)
RLI Continuation-in-part of Ser. No. US 729622 Continuation-in-part of Ser. No. US 1996-680574, filed on 12 Jul 1996 Continuation-in-part of Ser. No. US 1996-658800, filed on 5 Jun 1996 Continuation-in-part of Ser. No. US 1996-620280, filed on 22 Mar 1996, now abandoned Continuation-in-part of Ser. No. US 1995-532136, filed on 22 Sep 1995, now abandoned Continuation of Ser. No. US 1995-523435, filed on 1 Sep 1995, now abandoned
DT Utility
FS GRANTED
EXNAM Primary Examiner: Swartz, Rodney P.
LREP Townsend and Townsend and Crew LLP
CLMN Number of Claims: 93
ECL Exemplary Claim: 1

DRWN 11 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 2650
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compounds and methods for diagnosing ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of one or more M. ***tuberculosis*** proteins, and DNA sequences encoding such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of M. ***tuberculosis*** infection in patients and biological samples. Antibodies directed against such polypeptides are also provided.

TI Compounds and methods for diagnosis of ***tuberculosis***

AB Compounds and methods for diagnosing ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of one or more M. ***tuberculosis*** proteins, and DNA sequences encoding such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of M. ***tuberculosis*** infection in patients and biological samples. Antibodies directed against such polypeptides are also provided.

SUMM The present invention relates generally to the detection of Mycobacterium ***tuberculosis*** infection. The invention is more particularly related to polypeptides comprising a Mycobacterium ***tuberculosis*** antigen, or a portion or other variant thereof, and the use of such polypeptides for the serodiagnosis of Mycobacterium ***tuberculosis*** infection.

SUMM ***Tuberculosis*** is a chronic, infectious disease, that is generally caused by infection with Mycobacterium ***tuberculosis*** . It is a major disease in developing countries, as well as an increasing problem in developed areas of the world,. . .

SUMM Although ***tuberculosis*** can generally be controlled using extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease.. . .

SUMM Inhibiting the spread of ***tuberculosis*** will require effective vaccination and accurate, early diagnosis of the disease. Currently, vaccination with live bacteria is the most efficient. . .

SUMM While macrophages have been shown to act as the principal effectors of M. ***tuberculosis*** immunity, T cells are the predominant inducers of such immunity. The essential role of T cells in protection against M. ***tuberculosis*** infection is illustrated by the frequent occurrence of M. ***tuberculosis*** in AIDS patients, due to the depletion of CD4 T cells associated with human immunodeficiency virus (HIV) infection. Mycobacterium-reactive CD4. . . that 1,25-dihydroxy-vitamin D3, either alone or in combination with IFN-.gamma. or tumor necrosis factor-alpha, activates human macrophages to inhibit M. ***tuberculosis*** infection. Furthermore, it is known that IFN-.gamma. stimulates human macrophages to make 1,25-dihydroxy-vitamin D3. Similarly, IL-12 has been shown to play a role in stimulating resistance to M. ***tuberculosis*** infection. For a review of the immunology of M. ***tuberculosis*** infection see Chan and Kaufmann, in ***Tuberculosis*** : Pathogenesis, Protection and Control, Bloom (ed.), ASM Press, Washington, DC, 1994.

SUMM Accordingly, there is a need in the art for improved diagnostic methods for detecting ***tuberculosis*** . The present invention fulfills this need and further provides other related advantages.

SUMM Briefly stated, the present invention provides compositions and methods for diagnosing ***tuberculosis*** . In one aspect, polypeptides are provided comprising an antigenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. In one embodiment of. . .

SUMM In a related aspect, polypeptides are provided comprising an immunogenic portion of an M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, the antigen having one. . .

SUMM In a related aspect, the polypeptides comprise an antigenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, wherein the antigen comprises. . .

SUMM . . . provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, an inventive polypeptide and a known M. ***tuberculosis*** antigen.

SUMM In further aspects of the subject invention, methods and diagnostic kits are provided for detecting ***tuberculosis*** in a patient. The methods comprise: (a) contacting a biological sample with at least one of the above polypeptides; and (b) detecting in the sample the presence of antibodies that bind to the polypeptide or polypeptides, thereby detecting M. ***tuberculosis*** infection in the biological sample. Suitable biological samples include whole blood, sputum, serum, plasma, saliva, cerebrospinal fluid and urine. The. . .

SUMM The present invention also provides methods for detecting M. ***tuberculosis*** infection comprising: (a) obtaining a biological sample from a patient; (b) contacting the sample with at least one oligonucleotide primer. . .

SUMM In a further aspect, the present invention provides a method for detecting M. ***tuberculosis*** infection in a patient comprising: (a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide. . .

SUMM . . . monoclonal, that bind to the polypeptides described above, as well as methods for their use in the detection of M. ***tuberculosis*** infection.

DRWD . . . B illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a first and a second M. ***tuberculosis*** -immune donor, respectively, by the 14 Kd, 20 Kd and 26 Kd antigens described in Example 1.

DRWD FIGS. 2A-D illustrate the reactivity of antisera raised against secretory M. ***tuberculosis*** proteins, the known M. ***tuberculosis*** antigen 85b and the inventive antigens Tb38-1 and TbH-9, respectively, with M. ***tuberculosis*** lysate (lane 2), M. ***tuberculosis*** secretory proteins (lane 3), recombinant Tb38-1 (lane 4), recombinant TbH-9 (lane 5) and recombinant 85b (lane 5).

DRWD FIG. 3A illustrates the stimulation of proliferation in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, recombinant TbH-9 and a control antigen, TbRa11.

DRWD FIG. 3B illustrates the stimulation of interferon-y production in a TbH-9-specific F cell clone by secretory M. ***tuberculosis*** proteins, PPD and recombinant TbH-9.

DRWD FIG. 4 illustrates the reactivity of two representative polypeptides with sera from M. ***tuberculosis*** -infected and uninfected individuals, as compared to the reactivity of bacterial lysate.

DRWD FIG. 5 shows the reactivity of four representative polypeptides with sera from M. ***tuberculosis*** -infected and uninfected individuals, as compared to the reactivity of the 38 kD antigen.

DRWD FIG. 6 shows the reactivity of recombinant 38 kD and TbRa11 antigens with sera from M. ***tuberculosis*** patients, PPD positive donors and normal donors.

DRWD FIG. 8 shows the reactivity of the antigen of SEQ ID No. 60 with sera from M. ***tuberculosis*** patients and normal donors.

DRWD FIG. 9 illustrates the reactivity of the recombinant antigen TbH-29 (SEQ ID NO: 137) with sera from M. ***tuberculosis*** patients, PPD positive donors and normal donors as determined by indirect ELISA.

DRWD FIG. 10 illustrates the reactivity of the recombinant antigen TbH-33 (SEQ ID NO: 140) with sera from M. ***tuberculosis*** patients and from normal donors, and with a pool of sera from M. ***tuberculosis*** patients, as determined both by direct and indirect ELISA

DRWD . . . 11 illustrates the reactivity of increasing concentrations of the recombinant antigen TbH-33 (SEQ ID NO: 140) with sera from M. ***tuberculosis*** patients and from normal donors as determined by EILISA.

DRWD SEQ ID NO: 149 is the DNA sequence of the M. ***tuberculosis*** antigen 38 kD.

DRWD SEQ ID NO: 150 is the amino acid sequence of the M. ***tuberculosis*** antigen 38 kD.

DETD As noted above, the present invention is generally directed to compositions and methods for diagnosing ***tuberculosis*** . The compositions of the subject invention include polypeptides that comprise at least one antigenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. Polypeptides within the scope of the present invention include, but are not limited to, soluble M. ***tuberculosis*** antigens. A "soluble M. ***tuberculosis*** antigen" is a protein of M. ***tuberculosis*** origin that is present in M. ***tuberculosis*** culture filtrate. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e.,. . . entirely of the antigenic portion, or may contain additional sequences. The additional sequences may be derived from the native M. ***tuberculosis*** antigen or may be heterologous, and such sequences may (but need not) be antigenic.

DETD . . . may or may not be soluble) is a portion that is capable of reacting with sera obtained from an M. ***tuberculosis*** -infected individual (i.e., generates an absorbance reading with sera from infected individuals that is at least three standard deviations above the absorbance obtained with sera from uninfected individuals, in a representative ELISA assay described herein). An "M. ***tuberculosis*** -infected individual" is a human who has been infected with M. ***tuberculosis*** (e.g., has an intradermal skin test response to PPD that is at least 0.5 cm in diameter). Infected individuals may display symptoms of ***tuberculosis*** or may be free of disease symptoms. Polypeptides comprising at least an antigenic portion of one or more M. ***tuberculosis*** antigens as described herein may generally be used, alone or in combination, to detect ***tuberculosis*** in a patient.

DETD . . . polypeptide" is a polypeptide comprising at least one of the above antigenic portions and one or more additional antigenic M. ***tuberculosis*** sequences, which are joined via a peptide linkage

into a single amino acid chain. The sequences may be joined directly. . .

DETD In general, M. ***tuberculosis*** antigens, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, soluble antigens may be isolated from M. ***tuberculosis*** culture filtrate by procedures known to those of ordinary skill in the art, including anion-exchange and reverse phase chromatography. Purified. . . may then be evaluated for a desired property, such as the ability to react with sera obtained from an M. ***tuberculosis*** -infected individual. Such screens may be performed using the representative methods described herein. Antigens may then be partially sequenced using, for. . .

DETD . . . vector and expressed in an appropriate host. DNA molecules encoding soluble antigens may be isolated by screening an appropriate M. ***tuberculosis*** expression library with anti-sera (e.g., rabbit) raised specifically against soluble M. ***tuberculosis*** antigens. DNA sequences encoding antigens that may or may not be soluble may be identified by screening an appropriate M. ***tuberculosis*** genomic or cDNA expression library with sera obtained from patients infected with M. ***tuberculosis*** . Such screens may generally be performed using techniques well known in the art, such as those described in Sambrook et. . .

DETD DNA sequences encoding soluble antigens may also be obtained by screening an appropriate M. ***tuberculosis*** cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of. . .

DETD . . . antigens described herein are "antigenic." More specifically, the antigens have the ability to react with sera obtained from an M. ***tuberculosis*** -infected individual. Reactivity may be evaluated using, for example, the representative EILISA assays described herein, where an absorbance reading with sera. . .

DETD Antigenic portions of M. ***tuberculosis*** antigens may be prepared and identified using well known techniques, such as those summarized in Paul, Fundamental Immunology, 3d ed.,. . . is substantially similar to that generated by the full length antigen. In other words, an antigenic portion of a M. ***tuberculosis*** antigen generates at least about 20%, and preferably about 100%, of the signal induced by the full length antigen in. . .

DETD Portions and other variants of M. ***tuberculosis*** antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally. . .

DETD In certain specific embodiments, the subject invention discloses polypeptides comprising at least an antigenic portion of a soluble M. ***tuberculosis*** antigen (or a variant of such an antigen), where the antigen has one of the following N-terminal sequences:

DETD In a further specific embodiment, the subject invention discloses polypeptides comprising at least an immunogenic portion of an M. ***tuberculosis*** antigen having one of the following N-terminal sequences, or a variant thereof that differs only in conservative substitutions and/or modifications:

DETD In other specific embodiments, the subject invention discloses polypeptides comprising at least an antigenic portion of a soluble M. ***tuberculosis*** antigen (or a variant of such an antigen) that comprises one or more of the amino acid sequences encoded by. . .

DETD In further specific embodiments, the subject invention discloses

polypeptides comprising at least an antigenic portion of a M. ***tuberculosis*** antigen (or a variant of such an antigen), which may or may not be soluble, that comprises one or more. . .

DETD In the specific embodiments discussed above, the M. ***tuberculosis*** antigens include variants that are encoded DNA sequences which are substantially homologous to one or more of DNA sequences specifically. . .

DETD . . . comprising a first and a second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known M. ***tuberculosis*** antigen, such as the 38 kD antigen described above or ESAT-6 (SEQ ID Nos. 98 and 99), together with variants. . .

DETD In another aspect, the present invention provides methods for using the polypeptides described above to diagnose ***tuberculosis*** . In this aspect, methods are provided for detecting M. ***tuberculosis*** infection in a biological sample, using one or more of the above polypeptides, alone or in combination. In embodiments in. . . a predetermined cut-off value. The presence of such antibodies indicates previous sensitization to mycobacteria antigens which may be indicative of ***tuberculosis*** .

DETD . . . using each polypeptide individually to evaluate serum samples obtained from a series of patients known to be infected with M. ***tuberculosis*** . After determining which samples test positive (as described below) with each polypeptide, combinations of two or more polypeptides may be. . . infection in most, or all, of the samples tested. Such polypeptides are complementary. For example, approximately 25-30% of sera from ***tuberculosis*** -infected individuals are negative for antibodies to any single protein, such as the 38 kD antigen mentioned above. Complementary polypeptides may,. . .

DETD . . . (i.e., incubation time) is that period of time that is sufficient to detect the presence of antibody within a M. ***tuberculosis*** -infected sample. Preferably, the contact time is sufficient to achieve a level of binding that is at least 95% of that. . .

DETD To determine the presence or absence of anti-M. ***tuberculosis*** antibodies in the sample, the signal detected from the reporter group that remains bound to the solid support is generally. . . general, a sample generating a signal that is three standard deviations above the predetermined cut-off value is considered positive for ***tuberculosis*** . In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of. . . a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for ***tuberculosis*** .

DETD . . . reagent and to the area of immobilized polypeptide. Concentration of detection reagent at the polypeptide indicates the presence of anti-M. ***tuberculosis*** antibodies in the sample. Typically, the concentration of detection reagent at that site generates a pattern, such as a line,. . .

DETD Antibodies may be used in diagnostic tests to detect the presence of M. ***tuberculosis*** antigens using assays similar to those detailed above and other techniques well known to those of skill in the art, thereby providing a method for detecting M. ***tuberculosis*** infection in a patient.

DETD . . . example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify M.

tuberculosis -specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a DNA molecule. . .

DETD . . . art (see, for example, Mullis et al. Ibid; Ehrlich, Ibid). Primers or probes may thus be used to detect M. ***tuberculosis*** -specific sequences in biological samples. DNA probes or primers comprising oligonucleotide sequences described above may be used alone, in combination with. . .

DETD PURIFICATION AND CHARACTERIZATION OF POLYPEPTIDES FROM M. ***TUBERCULOSIS*** CULTURE FILTRATE

DETD This example illustrates the preparation of M. ***tuberculosis*** soluble polypeptides from culture filtrate. Unless otherwise noted, all percentages in the following example are weight per volume.

DETD M. ***tuberculosis*** (either H37Ra, ATCC No. 25177, or H37Rv, ATCC No. 25618) was cultured in sterile GAS media at 37.degree. C. for. . .

DETD Additional soluble antigens were isolated from M. ***tuberculosis*** culture filtrate as follows. M. ***tuberculosis*** culture filtrate was prepared as described above. Following dialysis against Bis-Tris propane buffer, at pH 5.5, fractionation was performed using. . .

DETD DNA sequences that encode the antigens designated as (a), (c), (d) and (g) above were obtained by screening a M. ***tuberculosis*** genomic library using .sup.32P end labeled degenerate oligonucleotides corresponding to the N-terminal sequence and containing M. ***tuberculosis*** codon bias. The screen performed using a probe corresponding to antigen (a) above identified a clone having the sequence provided. . .

DETD . . . was amplified from genomic DNA using the sequence obtained from GENBANK. This sequence was then used to screen an M. ***tuberculosis*** library and a full length copy of the M. ***tuberculosis*** homologue was obtained (SEQ ID No. 94).

DETD The amino acid sequence for antigen (j) was found to be homologous to a known M. ***tuberculosis*** protein translated from a DNA sequence. To the best of the inventors' knowledge, this protein has not been previously shown. . .

DETD USE OF PATIENT SERA TO ISOLATE M. ***TUBERCULOSIS*** ANTIGENS

DETD This example illustrates the isolation of antigens from M. ***tuberculosis*** lysate by screening with serum from M. ***tuberculosis*** -infected individuals.

DETD Dessicated M. ***tuberculosis*** H37Ba (Difco Laboratories) was added to a 2% NP40 solution, and alternately homogenized and sonicated three times. The resulting suspension. . . a Centriprep 10 (Amicon, Beverley, Mass.) and screened by Western blot for serological activity using a serum pool from M. ***tuberculosis*** -infected patients which was not immunoreactive with other antigens of the present invention.

DETD PREPARATION OF DNA SEQUENCES ENCOFDING M. ***TUBERCULOSIS*** ANTIGENS

DETD This example illustrates the preparation of DNA sequences encoding M. ***tuberculosis*** antigens by screening a M. ***tuberculosis*** expression library with sera obtained from patients infected with M. ***tuberculosis*** , or with anti-sera raised against M. ***tuberculosis*** antigens.

DETD A. PREPARATION OF M. ***TUBERCULOSIS*** SOLUBLE ANTIGENS USING RABBIT ANTI-SERA

DETD Genomic DNA was isolated from the M. ***tuberculosis*** strain

H37Ra. The DNA was randomly sheared and used to construct an expression library using the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Rabbit anti-sera was generated against secretory proteins of the M. ***tuberculosis*** strains H37Ra, H37Rv and Erdman by immunizing a rabbit with concentrated supernatant of the M. ***tuberculosis*** cultures. Specifically, the rabbit was first immunized subcutaneously with 200 .mu.g of protein antigen in a total volume of 2. . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD Thirty two clones were purified. Of these, 25 represent sequences that have not been previously identified in M. ***tuberculosis*** . Proteins were induced by IPTG and purified by gel elution, as described in Skeiky et al., J. Exp. Med. 181:1527-1537,. . .

DETD . . . ID Nos. 77, 69, 71, 76) show some homology to sequences previously identified in Mycobacterium leprae but not in M. ***tuberculosis*** . TbRA11, TbRA26, TbRA28 and TbDPEP (SEQ ID Nos. 66, 74, 75, 53) have been previously identified in M. ***tuberculosis*** . No significant homologies were found to TbRA1, TbRA3, TbRA4, TbRA9, TbRA10, TbRA13, TbRA17, TbRA19, TbRA29, TbRA32, TbRA36 and the overlapping. . .

DETD B. USE OF PATIENT SERA TO IDENTIFY DNA SEQUENCES ENCODING M. ***TUBERCULOSIS*** ANTIGENS

DETD . . . DNA library described above, and an additional H37Rv library, were screened using pools of sera obtained from patients with active ***tuberculosis*** . To prepare the H37Rv library, M. ***tuberculosis*** strain H37Rv genomic DNA was isolated, subjected to partial Sau3A digestion and used to construct an expression library using the. . . and TbH=high reactivity) in both ELISA and immunoblot format. A fourth pool of sera from seven patients with active pulmonary ***tuberculosis*** was also employed. All of the sera lacked increased reactivity with the recombinant 38 kD M. ***tuberculosis*** H37Ra phosphate-binding protein.

DETD . . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD Thirty two clones were purified. Of these, 31 represented sequences that had not been previously identified in human M. ***tuberculosis*** . Representative sequences of the DNA molecules identified are provided in SEQ ID NOS.: 26-51 and 100. Of these, TbH-8-2 (SEQ.. . . of the open reading frame for the antigen ESAT-6 previously identified in M. bovis (Acc. No. U34848) and in M. ***tuberculosis*** (Sorensen et al., Infec. Immun. 63:1710-1717, 1995).

DETD . . . sequence of Tb38-1F3 is presented in SEQ. ID. NO. 112. A Tbl-1-9 probe identified three clones in the H37Rv library: ***TbH*** - ***9*** - ***FL*** (SEQ. ID NO. 101), which may be the homologue of TbH-9 (R37Ra), TbH-9-1 (SEQ. ID NO. 103), and TbH-8-2 (SEQ.. . .

DETD Further screening of the M. ***tuberculosis*** genomic DNA library, as described above, resulted in the recovery of ten additional reactive clones, representing seven different genes. One. . . determined to be identical to the 14 Kd alpha crystalline heat shock protein previously shown to be present in M. ***tuberculosis*** , and a third was determined to be identical to the antigen TbH-8 described above. The determined DNA sequences for the. . . contains the reactive open reading frame), although the 3' end of TbH-29 was found to be identical

to the M. ***tuberculosis*** cosinid Y227. TbH-32 and TbH-33 were found to be identical to the previously identified M. ***tuberculosis*** insertion element IS6110 and to the M. ***tuberculosis*** cosmid Y50, respectively. No significant homologies to TbH-30 were found.

DETD . . . and uninduced lysates were run in duplicate on SDS-PAGE and transferred to nitrocellulose filters. Filters were reacted with human M. ***tuberculosis*** sera (1:200 dilution) reactive with TbH and a rabbit sera (1:200 or 1:250 dilution) reactive with the N-terminal 4 Kd. . .

DETD Positive reaction of the recombinant human M. ***tuberculosis*** antigens with both the human M. ***tuberculosis*** sera and znti-lacZ sera indicate that reactivity of the human M. ***tuberculosis*** sera is directed towards the fusion protein. Antigens reactive with the anti-lacZ sera but not with the human M. ***tuberculosis*** sera may be the result of the human M. ***tuberculosis*** sera recognizing conformational epitopes, or the antigen-antibody binding kinetics may be such that the 2 hour sera exposure in the. . .

DETD Studies were undertaken to determine whether the antigens TbH-9 and Tb38-1 represent cellular proteins or are secreted into M. ***tuberculosis*** culture media. In the first study, rabbit sera were raised against A) secretory proteins of M. ***tuberculosis*** , B) the known secretory recombinant M. ***tuberculosis*** antigen 85b, C) recombinant Tb38-1 and D) recombinant TbH-9, using protocols substantially as described in Example 3A. Total M. ***tuberculosis*** lysate, concentrated supernatant of M. ***tuberculosis*** cultures and the recombinant antigens 85b, TbH-9 and Tb38-1 were resolved on denaturing gels, immobilized on nitrocellulose membranes and duplicate. . .

DETD . . . FIGS. 2A-D, respectively, wherein the lane designations are as follows: 1) molecular weight protein standards; 2) 5 tg of M ***tuberculosis*** lysate; 3) 5 .mu.g secretory proteins; 4) 50 ng recombinant Tb38-1; 5) 50 ng recombinant TbH-9; and 6) 50 ng. . . by an arrow). These results demonstrate that Tb38-1 and TbH-9 are intracellular antigens and are not actively secreted by M. ***tuberculosis*** .

DETD . . . an intracellular antigen was confirmed by determining the reactivity of TbH-9-specific human T cell clones to recombinant TbH-9, secretory M. ***tuberculosis*** proteins and PPD. A TbH-9-specific T cell clone (designated 131TbH-9) was generated from PB,MC of a healthy PPD-positive donor. The proliferative response of 131 TbH-9 to secretory proteins, recombinant TbH-9 and a control M. ***tuberculosis*** antigen, TbRa11, was determined by measuring uptake of tritiated thymidine, as described in Example 1. As shown in FIG. 3A, the clone 131TbH-9 responds specifically to TbH-9, showing that TbH-9 is not a significant component of M. ***tuberculosis*** secretory proteins. FIG. 3B shows the production of IFN-.gamma. by a second TbH-9-specific T cell clone (designated PPD 800-10) prepared. . . cell clone with secretory proteins, PPD or recombinant TbH-9. These results further confirm that TbH-9 is not secreted by M. ***tuberculosis*** .

DETD An M. ***tuberculosis*** polypeptide was isolated from tuberculin purified protein derivative (PPD) as follows.

DETD . . . F. et al., Tuberculin purified protein derivative. Prepiration and analyses of a large quantity for standard. The American Review of

Tuberculosis 44:9-25, 1941). M. ***tuberculosis*** Rv strain was grown for 6 weeks in synthetic medium in roller bottles at 37.degree. C. Bottles containing the bacterial. . .

DETD Six fractions were collected, dried, suspended in PBS and tested individually in M. ***tuberculosis*** -infected guinea pigs for induction of delayed type hypersensitivity (DTH) reaction. One fraction was found to induce a strong DTH reaction. . . 80 .mu.l/minute. Eluent was monitored at 215 nm. Eight fractions were collected and tested for induction of DTH in M. ***tuberculosis*** -infected guinea pigs. One fraction was found to induce strong DTH of about 16 mm induration. The other fractions did not. . .

DETD USE OF REPRESENTATIVE ANTIGENS FOR SERODIAGNOSIS OF ***TUBERCULOSIS***

DETD . . . ELISA reactivity of two recombinant antigens isolated using method A in Example 3 (TbRa3 and TbRa9) with sera from M. ***tuberculosis*** positive and negative patients. The reactivity of these antigens is compared to that of bacterial lysate isolated from M. ***tuberculosis*** strain H37Ra (Difco, Detroit, Mich.). In both cases, the recombinant antigens differentiated positive from negative sera. Based on cut-off values. . .

DETD The reativity of four antigens (TbRa3, TbRa9, TbH4 and TbH12) with sera from a group of M. ***tuberculosis*** infected patients with differing reactivity in the acid fast stain of sputum (Smithwick and David, Tubercle 52:226, 1971) was also examined, and compared to the reactivity of M. ***tuberculosis*** lysate and the 38 kD antigen. The results are presented in Table 3, below:

DETD

TABLE 3

REACTIVITY OF ANTIGENS WITR SERA FROM M. ***TUBERCULOSIS*** PATIENTS

Acid Fast		ELISA Values				
Patient	Sputum	Lysate	38kD	TbRa9	TbH12	TbH4	TbRa3
Tb01B93I-2	++++	1.853	0.634	0.998	1.022	1.030	1.314
Tb01B93I-19	++++. . .						

DETD . . . sensitivity of 27 out of 27, indicating that these antigens should complement each other in the serological detection of M. ***tuberculosis*** infection. In addition, several of the recombinant antigens detected positive sera that were not detected using the 38 kD antigen,. . .

DETD The reactivity of the recombinant antigen TbRa11 with sera from M. ***tuberculosis*** patients shown to be negative fcr the 38 kD antigen, as well as with sera from PPD positive and normal. . .

DETD . . . After washing, the assay was developed with TMB substrate as described above. The reactivity of TbRa2A with sera from M. ***tuberculosis*** patients and normal donors in shown in Table 4. The mean value for reactivity of TbRa2A with sera from M. ***tuberculosis*** patients was 0.444 with a standard deviation of 0.309. The mean for reactivity with sera from normal donors was 0.109. . .

DETD

TABLE 4

REACTIVITY OF TBRA2A WITH SERA FROM M. ***TUBERCULOSIS*** PATIENTS AND FROM NORMAL DONORS

Serum ID Status OD 450

Tb85 TB 0.680
Tb86 TB 0.450
Tb87 TB 0.263
Th88 TB 0.275
Tb89 TB 0.403
Tb91 TB 0.393
Tb92. . .
DETD The reactivity of the recombinant antigen (g) (SEQ ID No. 60) with sera from M. ***tuberculosis*** patients and normal donors was determined by ELISA as described above. FIG. 8 shows the results o f the titration of antigen (g) with four M. ***tuberculosis*** positive sera that were all reactive with the 38 kD antigen and with four donor sera. All four positive sera. . .
DETD The reactivity of the recombinant antigen TbH-29 (SEQ ID NO: 137) with sera from M. ***tuberculosis*** patients, PPD positive donors and normal donors was determined by indirect ELISA as described above. The results are shown in FIG. 9. TbH-29 detected 30 out of 60 M. ***tuberculosis*** sera, 2 out of 8 PPD positive sera and 2 out of 27 normal sera.
DETD . . . results of ELISA tests (both direct and indirect) of the antigen TbH-33 (SEQ ID NO: 140) with sera from M. ***tuberculosis*** patients and from normal donors and with a pool of sera from M. ***tuberculosis*** patients. The mean OD 450 was demonstrated to be higher with sera from M. ***tuberculosis*** patients than from normal donors, with the mean OD 450 being significantly higher in the indirect ELISA than in the direct ELISA. FIG. 11 is a titration curve for the reactivity of recombinant TbH-33 with sera from M. ***tuberculosis*** patients and from normal donors showing an increase in OD 450 with increasing concentration of antigen.
DETD PREPARATION AND CHARACTERIZATION OF M. ***TUBERCULOSIS*** FUSION PROTEINS
DETD USE OF M. ***TUBERCULOSIS*** FUSION PROTEINS FOR SERODIAGNOSIS OF ***TUBERCULOSIS***
DETD The effectiveness of the fusion protein TbRa3-38 kD-Tb38-1, prepared as described above, in the serodiagnosis of ***tuberculosis*** infection was examined by ELISA.
DETD . . . 6, with the fusion protein being coated at 200 ng/well. A panel of sera was chosen from a group of ***tuberculosis*** patients previously shown, either by ELISA or by western blot analysis, to react with each of the three antigens individually. . .
DETD
TABLE 5

REACTIVITY OF FUSION PROTEIN
WITH SERA FROM M. ***TUBERCULOSIS*** PATIENTS
ELISA and/or Western Blot Fusion Fusion
Reactivity with Individual proteins recombinant Recombinant
Serum ID Status 38kd Tb38-1 TbRa3 OD 450. . .

L15 ANSWER 7 OF 7 USPATFULL on STN
AN 2001:157807 USPATFULL
TI Compounds and methods for immunotherapy and diagnosis of ***tuberculosis***

IN Reed, Steven G., Bellevue, WA, United States
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PA Corixa Corporation, Seattle, WA, United States (U.S. corporation)
PI US 6290969 B1 20010918
AI US 1997-818112 19970313 (8)
RLI Continuation-in-part of Ser. No. US 1996-730510, filed on 11 Oct 1996 Continuation-in-part of Ser. No. US 1996-680574, filed on 12 Jul 1996 Continuation-in-part of Ser. No. US 1996-659683, filed on 5 Jun 1996 Continuation-in-part of Ser. No. US 1996-620874, filed on 22 Mar 1996, now abandoned Continuation-in-part of Ser. No. US 1995-533634, filed on 22 Sep 1995, now abandoned Continuation-in-part of Ser. No. US 1995-523436, filed on 1 Sep 1995, now abandoned
DT Utility
FS GRANTED
EXNAM Primary Examiner: Swartz, Rodney P.
LREP Townsend & Townsend & Crew LLP
CLMN Number of Claims: 98
ECL Exemplary Claim: 1
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CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Compounds and methods for inducing protective immunity against ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one immunogenic portion of one or more M. ***tuberculosis*** proteins and DNA molecules encoding such polypeptides. Such compounds may be formulated into vaccines and/or pharmaceutical compositions for immunization against M. ***tuberculosis*** infection, or may be used for the diagnosis of ***tuberculosis*** .
TI Compounds and methods for immunotherapy and diagnosis of ***tuberculosis***
AB Compounds and methods for inducing protective immunity against ***tuberculosis*** are disclosed. The compounds provided include polypeptides that contain at least one immunogenic portion of one or more M. ***tuberculosis*** proteins and DNA molecules encoding such polypeptides. Such compounds may be formulated into vaccines and/or pharmaceutical compositions for immunization against M. ***tuberculosis*** infection, or may be used for the diagnosis of ***tuberculosis*** .
SUMM The present invention relates generally to detecting, treating and preventing Mycobacterium ***tuberculosis*** infection. The invention is more particularly related to polypeptides comprising a Mycobacterium ***tuberculosis*** antigen, or a portion or other variant thereof, and the use of such polypeptides for diagnosing and vaccinating against Mycobacterium ***tuberculosis*** infection.
SUMM ***Tuberculosis*** is a chronic, infectious disease, that is generally caused by infection with Mycobacterium ***tuberculosis*** . It is a major disease in developing countries, as well as an increasing problem in developed areas of the world,. . .
SUMM Although ***tuberculosis*** can generally be controlled using

extended antibiotic therapy, such treatment is not sufficient to prevent the spread of the disease.. . .

SUMM Inhibiting the spread of ***tuberculosis*** requires effective vaccination and accurate, early diagnosis of the disease. Currently, vaccination with live bacteria is the most efficient method. . .

SUMM While macrophages have been shown to act as the principal effectors of M. ***tuberculosis*** immunity, T cells are the predominant inducers of such immunity. The essential role of T cells in protection against M. ***tuberculosis*** infection is illustrated by the frequent occurrence of M. ***tuberculosis*** in AIDS patients, due to the depletion of CD4 T cells associated with human immunodeficiency virus (HIV) infection. Mycobacterium-reactive CD4. . . that 1,25-dihydroxy-vitamin D3, either alone or in combination with IFN-.gamma. or tumor necrosis factor-alpha, activates human macrophages to inhibit M. ***tuberculosis*** infection. Furthermore, it is known that IFN-.gamma. stimulates human macrophages to make 1,25-dihydroxy-vitamin D3. Similarly, IL-12 has been shown to play a role in stimulating resistance to M. ***tuberculosis*** infection. For a review of the immunology of M. ***tuberculosis*** infection see Chan and Kaufmann in ***Tuberculosis*** : Pathogenesis, Protection and Control, Bloom (ed.), ASM Press, Washington, DC, 1994.

SUMM Accordingly, there is a need in the art for improved vaccines and methods for preventing, treating and detecting ***tuberculosis*** . The present invention fulfills these needs and further provides other related advantages.

SUMM Briefly stated, this invention provides compounds and methods for preventing and diagnosing ***tuberculosis*** . In one aspect, polypeptides are provided comprising an immunogenic portion of a soluble M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. In one embodiment of. . .

SUMM In a related aspect, polypeptides are provided comprising an immunogenic portion of an M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, the antigen having one. . .

SUMM In a related aspect, the polypeptides comprise an immunogenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications, wherein the antigen comprises. . .

SUMM . . . provides fusion proteins comprising a first and a second inventive polypeptide or, alternatively, an inventive polypeptide and a known M. ***tuberculosis*** antigen.

SUMM In further aspects of this invention, methods and diagnostic kits are provided for detecting ***tuberculosis*** in a patient. The methods comprise contacting dermal cells of a patient with one or more of the above polypeptides. . .

SUMM In yet other aspects, methods are provided for detecting ***tuberculosis*** in a patient, such methods comprising contacting dermal cells of a patient with one or more polypeptides encoded by a. . .

DRWD . . . B illustrate the stimulation of proliferation and interferon-.gamma. production in T cells derived from a first and a second M. ***tuberculosis*** -immune donor, respectively, by the 14 Kd, 20 Kd and 26 Kd antigens described in Example 1.

DRWD FIG. 2 illustrates the stimulation of proliferation and

interferon-.gamma. production in T cells derived from an M. ***tuberculosis*** -immune individual by the two representative polypeptides TbRa3 and ThRa9.

DRWD FIGS. 3A-D illustrate the reactivity of antisera raised against secretory M. ***tuberculosis*** proteins, the known M. ***tuberculosis*** antigen 85b and the inventive antigens Tb38-1 and TbH-9, respectively, with M. ***tuberculosis*** lysate (lane 2), M. ***tuberculosis*** secretory proteins (lane 3), recombinant Tb38-1 (lane 4), recombinant TbH-9 (lane 5) and recombinant 85b (lane 5).

DRWD FIG. 4A illustrates the stimulation of proliferation in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, recombinant TbH-9 and a control antigen, TbRa11.

DRWD FIG. 4B illustrates the stimulation of interferon-.gamma. production in a TbH-9-specific T cell clone by secretory M. ***tuberculosis*** proteins, PPD and recombinant TbH-9.

DRWD SEQ ID NO: 154 is the DNA sequence of the M. ***tuberculosis*** antigen 38 kD.

DRWD SEQ ID NO: 155 is the amino acid sequence of the M. ***tuberculosis*** antigen 38 kD.

DRWD As noted above, the present invention is generally directed to compositions and methods for preventing, treating and diagnosing ***tuberculosis*** . The compositions of the subject invention include polypeptides that comprise at least one immunogenic portion of a M. ***tuberculosis*** antigen, or a variant of such an antigen that differs only in conservative substitutions and/or modifications. Polypeptides within the scope of the present invention include, but are not limited to, immunogenic soluble M. ***tuberculosis*** antigens. A "soluble M. ***tuberculosis*** antigen" is a protein of M. ***tuberculosis*** origin that is present in M. ***tuberculosis*** culture filtrate. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins (i.e.,. . . . entirely of the immunogenic portion, or may contain additional sequences. The additional sequences may be derived from the native M. ***tuberculosis*** antigen or may be heterologous, and such sequences may (but need not) be immunogenic.

DRWD . . . from the group of T cells, NK cells, B cells and macrophages, where the cells are derived from an M. ***tuberculosis*** -immune individual. Polypeptides comprising at least an immunogenic portion of one or more M. ***tuberculosis*** antigens may generally be used to detect ***tuberculosis*** or to induce protective immunity against ***tuberculosis*** in a patient.

DRWD . . . polypeptide" is a polypeptide comprising at least one of the above immunogenic portions and one or more additional immunogenic M. ***tuberculosis***

DRWD In general, M. ***tuberculosis*** antigens, and DNA sequences encoding such antigens, may be prepared using any of a variety of procedures. For example, soluble antigens may be isolated from M. ***tuberculosis*** culture filtrate by procedures known to those of ordinary skill in the art, including anion-exchange and reverse phase chromatography. Purified. . .

DRWD . . . vector and expressed in an appropriate host. DNA molecules encoding soluble antigens may be isolated by screening an appropriate M. ***tuberculosis*** expression library with anti-sera (e.g., rabbit) raised specifically against soluble M. ***tuberculosis*** antigens. DNA sequences encoding antigens that may or may not be soluble may be

identified by screening an appropriate M. ***tuberculosis*** genomic or cDNA expression library with sera obtained from patients infected with M. ***tuberculosis*** . Such screens may generally be performed using techniques well known to those of ordinary skill in the art, such as. . .

DRWD DNA sequences encoding soluble antigens may also be obtained by screening an appropriate M. ***tuberculosis*** cDNA or genomic DNA library for DNA sequences that hybridize to degenerate oligonucleotides derived from partial amino acid sequences of. . .

DRWD Alternatively, genomic or cDNA libraries derived from M. ***tuberculosis*** may be screened directly using peripheral blood mononuclear cells (PBMCs) or T cell lines or clones derived from one or more M. ***tuberculosis*** -immune individuals. In general, PBMCs and/or T cells for use in such screens may be prepared as described below. Direct library. . . of expressed recombinant proteins for the ability to induce proliferation and/or interferon-.gamma. production in T cells derived from an M. ***tuberculosis*** -immune individual. Alternatively, potential T cell antigens may be first selected based on antibody reactivity, as described above.

DRWD . . . production (i e., interferon-.gamma. and/or interleukin-12 production) in T cells, NK cells, B cells and/or macrophages derived from an M. ***tuberculosis*** -immune individual. The selection of cell type for use in evaluating an immunogenic response to a antigen will, of course, depend. . . the desired response. For example, interleukin-12 production is most readily evaluated using preparations containing B cells and/or macrophages. An M. ***tuberculosis*** -immune individual is one who is considered to be resistant to the development of ***tuberculosis*** by virtue of having mounted an effective T cell response to M. ***tuberculosis*** (i.e., substantially free of disease symptoms). Such individuals may be identified based on a strongly positive (i.e., greater than about 10 mn diameter induration) intradermal skin test response to ***tuberculosis*** proteins (PPD) and an absence of any signs or symptoms of ***tuberculosis*** disease. T cells, NK cells, B cells and macrophages derived from M. ***tuberculosis*** -immune individuals may be prepared using methods known to those of ordinary skill in the art. For example, a preparation of. . . individual mycobacterial proteins, may be employed. Such T cell clones may be generated by, for example, culturing PBMCs from M. ***tuberculosis*** -immune individuals with mycobacterial proteins for a period of 2-4 weeks. This allows expansion of only the mycobacterial protein-specific T cells,. . . production (i.e., interferon-.gamma. and/or interleukin-12 production) performed using T cells, NK cells, B cells and/or macrophages derived from an M. ***tuberculosis*** -immune individual are considered immunogenic. Such assays may be performed, for example, using the representative procedures described below. Immunogenic portions of. . .

DRWD . . . and/or interleukin-12 production) in T cells, NK cells, B cells and/or macrophages derived from at least about 25% of M. ***tuberculosis*** -immune individuals. Among these immunogenic antigens, polypeptides having superior therapeutic properties may be distinguished based on the magnitude of the responses. . . proliferation and/or cytokine production in vitro in cells derived from more than about 25% of individuals that are not M. ***tuberculosis*** -immune, thereby eliminating responses that are not specifically due to

M. ***tuberculosis*** -responsive cells. Those antigens that induce a response in a high percentage of T cell, NK cell, B cell and/or macrophage preparations from M. ***tuberculosis*** -immune individuals (with a low incidence of responses in cell preparations from other individuals) have superior therapeutic properties.

DRWD Antigens with superior therapeutic properties may also be identified based on their ability to diminish the severity of M. ***tuberculosis*** infection in experimental animals, when administered as a vaccine. Suitable vaccine preparations for use on experimental animals are described in. . .

DRWD . . . identified based on the ability to elicit a response in an intradermal skin test performed on an individual with active ***tuberculosis*** , but not in a test performed on an individual who is not infected with M. ***tuberculosis*** . Skin tests may generally be performed as described below, with a response of at least 5 mm induration considered positive.

DRWD Portions and other variants of M. ***tuberculosis*** antigens may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally. . .

DRWD In certain specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a soluble M. ***tuberculosis*** antigen having one of the following N-terminal sequences, or a variant thereof that differs only in conservative substitutions and/or modifications:

DRWD In a further specific embodiment, the subject invention discloses polypeptides comprising at least an immunogenic portion of an M. ***tuberculosis*** antigen having one of the following N-terminal sequences, or a variant thereof that differs only in conservative substitutions and/or modifications:

DRWD In other specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a soluble M. ***tuberculosis*** antigen (or a variant of such an antigen) that comprises one or more of the amino acid sequences encoded by. . .

DRWD In further specific embodiments, the subject invention discloses polypeptides comprising at least an immunogenic portion of a M. ***tuberculosis*** antigen (or a variant of such an antigen), which may or may not be soluble, that comprises one or more. . .

DRWD In the specific embodiments discussed above, the M. ***tuberculosis*** antigens include variants that are encoded by DNA sequences which are substantially homologous to one or more of DNA sequences. . .

DRWD . . . comprising a first and a second inventive polypeptide or, alternatively, a polypeptide of the present invention and a known M ***tuberculosis*** antigen, such as the 38 kD antigen described in Andersen and Hansen, Infect. Immun. 57:2481-2488, 1989, (Genbank Accession No. M30046). . .

DRWD . . . or more of the above polypeptides or fusion proteins (or DNA molecules encoding such polypeptides) to induce protective immunity against ***tuberculosis*** in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be. . . may be free of detectable disease and/or infection. In other words, protective immunity may be induced to prevent or treat ***tuberculosis*** .

DRWD . . . adjuvant or a liposome (into which the polypeptide is incorporated). Such pharmaceutical compositions and vaccines may also contain other M. ***tuberculosis*** antigens, either incorporated

into a combination polypeptide or present within a separate polypeptide.

DRWD . . . above may be administered simultaneously with or sequentially to either a polypeptide of the present invention or a known M. ***tuberculosis*** antigen, such as the 38 kD antigen described above. For example, administration of DNA encoding a polypeptide of the present. . .

DRWD . . . described above, is capable of raising an immune response in an immunized patient sufficient to protect the patient from M. ***tuberculosis*** infection for at least 1-2 years. In general, the amount of polypeptide present in a dose (or produced in situ. . .

DRWD . . . aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune responses, such as lipid A, Bortadella pertussis or Mycobacterium ***tuberculosis*** . Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Freund's Complete Adjuvant (Difco Laboratories) and Merck Adjuvant. .

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DRWD In another aspect, this invention provides methods for using one or more of the polypeptides described above to diagnose ***tuberculosis*** using a skin test. As used herein, a "skin test" is any assay performed directly on a patient in which. . .

DRWD . . . than about 0.5 cm in diameter, preferably greater than about 1.0 cm in diameter, is a positive response, indicative of ***tuberculosis*** infection, which may or may not be manifested as an active disease.

DETD Purification and Characterization of Polypeptides FROM M. ***Tuberculosis*** Culture Filtrate

DETD This example illustrates the preparation of M. ***tuberculosis*** soluble polypeptides from culture filtrate. Unless otherwise noted, all percentages in the following example are weight per volume.

DETD M. ***tuberculosis*** (either H37Ra, ATCC No. 25177, or H37Rv, ATCC No. 25618) was cultured in sterile GAS media at 37.degree. C. for. . .

DETD Additional soluble antigens were isolated from M. ***tuberculosis*** culture filtrate as follows. M. ***tuberculosis*** culture filtrate was prepared as described above. Following dialysis against Bis-Tris propane buffer, at pH 5.5, fractionation was performed using. . .

DETD . . . sequences that encode the antigens designated as (a), (c), (d) and (g) above were obtained by screening a genomic M. ***tuberculosis*** library using .sup.32 P end labeled degenerate oligonucleotides corresponding to the N-terminal sequence and containing M. ***tuberculosis*** codon bias. The screen performed using a probe corresponding to antigen (a) above identified a clone having the sequence provided. . .

DETD . . . was amplified from genomic DNA using the sequence obtained from GENBANK. This sequence was then used to screen the M. ***tuberculosis*** library described below in Example 2 and a full length copy of the M. ***tuberculosis*** homologue was obtained (SEQ ID No. 99).

DETD The amino acid sequence for antigen (j) was found to be homologous to a known M. ***tuberculosis*** protein translated from a DNA sequence. To the best of the inventors' knowledge, this protein has not been previously shown. . .

DETD Use of Patient Sera to Isolate M. ***Tuberculosis*** Antigens

DETD This example illustrates the isolation of antigens from M. ***tuberculosis*** lysate by screening with serum from M. ***tuberculosis*** -infected individuals.

DETD Dessicated M. ***tuberculosis*** H37Ra (Difco Laboratories) was added to a 2% NP40 solution, and alternately homogenized and sonicated three times. The resulting suspension. . . Centriprep 10 (Amicon, Beverley, Mass.) and then screened by Western blot for serological activity using a serum pool from M. ***tuberculosis*** -infected patients which was not immunoreactive with other antigens of the present invention.

DETD Preparation of DNA Sequences Encoding M. ***tuberculosis*** Antigens

DETD This example illustrates the preparation of DNA sequences encoding M. ***tuberculosis*** antigens by screening a M. ***tuberculosis*** expression library with sera obtained from patients infected with M. ***tuberculosis*** , or with anti-sera raised against soluble M. ***tuberculosis*** antigens.

DETD A. PREPARATION OF M. ***TUBERCULOSIS*** SOLUBLE ANTIGENS USING RABBIT ANTI-SERA

DETD Genomic DNA was isolated from the M. ***tuberculosis*** strain H37Ra. The DNA was randomly sheared and used to construct an expression library using the Lambda ZAP expression system (Stratagene, La Jolla, Calif.). Rabbit anti-sera was generated against secretory proteins of the M. ***tuberculosis*** strains H37Ra, H37Rv and Erdman by immunizing a rabbit with concentrated supernatant of the M. ***tuberculosis*** cultures. Specifically, the rabbit was first immunized subcutaneously with 200 .mu.g of protein antigen in a total volume of 2. . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD Thirty two clones were purified. Of these, 25 represent sequences that have not been previously identified in human M. ***tuberculosis*** . Recombinant antigens were expressed and purified antigens used in the immunological analysis described in Example 1. Proteins were induced by. . .

DETD . . . ID Nos. 76, 68, 70, 75) show some homology to sequences previously identified in Mycobacterium leprae but not in M. ***tuberculosis*** . ThRA11, ThRA26, ThRA28 and TbDPEP (SEQ ID Nos.: 65, 73, 74, 53) have been previously identified in M. ***tuberculosis*** . No significant homologies were found to ThRA1, ThRA3, ThRA4, TbRA9, TBRA10, TbRA13, TbRA17, TbRal9, ThRA29, ThRA32, TbRA36 and the overlapping. . .

DETD The results of PBMC proliferation and interferon-.gamma. assays performed on representative recombinant antigens, and using T-cell preparations from several differentM. ***tuberculosis*** -immune patients, are presented in Tables 2 and 3, respectively.

DETD These results indicate that these soluble antigens can induce proliferation and/or interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** -immune individual.

DETD B. USE OF PATIENT SERA TO IDENTIFY DNA SEQUENCES ENCODING M. ***TUBERCULOSIS*** ANTIGENS

DETD . . . DNA library described above, and an additional H37Rv library, were screened using pools of sera obtained from patients with active ***tuberculosis*** . To prepare the H37Rv library, M. ***tuberculosis*** strain H37Rv genomic DNA was isolated, subjected to partial Sau3A digestion and used to construct an expression library using the. . . and TbH=high reactivity) in both ELISA and immunoblot format. A fourth pool of sera from seven patients with active pulmonary ***tuberculosis*** was also employed. All of the sera lacked increased

reactivity with the recombinant 38 kD M. ***tuberculosis*** H37Ra phosphate-binding protein.

DETD . . . Bacteriophage plaques expressing immunoreactive antigens were purified. Phagemid from the plaques was rescued and the nucleotide sequences of the M. ***tuberculosis*** clones deduced.

DETD Thirty two clones were purified. Of these, 31 represented sequences that had not been previously identified in human M. ***tuberculosis*** . Representative sequences of the DNA molecules identified are provided in SEQ ID Nos.: 26-51 and 105. Of these, TbH-8-2 (SEQ.. . . of the open reading frame for the antigen ESAT-6 previously identified in M. bovis (Acc. No. U34848) and in M. ***tuberculosis*** (Sorensen et al., Infec. Immun. 63:1710-1717, 1995).

DETD . . . sequence of Tb38-1F3 is presented in SEQ. ID. NO. 117. A TbH-9 probe identified three clones in the H37Rv library: ***TbH*** - ***9*** - ***FL*** (SEQ. ID NO. 106), which may be the homologue of TbH-9 (R37Ra), TbH-9-1 (SEQ. ID NO. 108), and TbH-9-4 (SEQ.. . .

DETD Further screening of the M. ***tuberculosis*** genomic DNA library, as described above, resulted in the recovery of ten additional reactive clones, representing seven different genes. One. . . was determined to be identical to the 14Kd alpha crystalline heat shock protein previously shown to be present in M. ***tuberculosis*** , and a third was determined to be identical to the antigen TbH-8 described above. The determined DNA sequences for the. . . contains the reactive open reading frame), although the 3' end of TbH-29 was found to be identical to the M. ***tuberculosis*** cosmid Y227. TbH-32 and TbH-33 were found to be identical to the previously identified M. ***tuberculosis*** insertion element IS6110 and to the M. ***tuberculosis*** cosmid Y50, respectively. No significant homologies to TbH-30 were found.

DETD . . . and uninduced lysates were run in duplicate on SDS-PAGE and transferred to nitrocellulose filters. Filters were reacted with human M. ***tuberculosis*** sera (1:200 dilution) reactive with TbH and a rabbit sera (1:200 or 1:250 dilution) reactive with the N-terminal 4 Kd. . .

DETD Positive reaction of the recombinant human M. ***tuberculosis*** antigens with both the human M. ***tuberculosis*** sera and anti-lacZ sera indicate that reactivity of the human M. ***tuberculosis*** sera is directed towards the fusion protein. Antigens reactive with the anti-lacZ sera but not with the human M. ***tuberculosis*** sera may be the result of the human M. ***tuberculosis*** sera recognizing conformational epitopes, or the antigen-antibody binding kinetics may be such that the 2 hour sera exposure in the. . .

DETD These results indicate that both the inventive M. ***tuberculosis*** antigens and ESAT-6 can induce proliferation and/or interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** -immune individual. To the best of the inventors' knowledge, ESAT-6 has not been previously shown to stimulate human immune responses

DETD . . . help to localize T-cell epitopes within Tb38-1 capable of inducing proliferation and interferon-.gamma. production in T-cells derived from an M. ***tuberculosis*** immune individual.

DETD Studies were undertaken to determine whether the antigens TbH-9 and Tb38-1 represent cellular proteins or are secreted into M. ***tuberculosis*** culture media. In the first study, rabbit sera were raised against A) secretory proteins of M. ***tuberculosis*** , B)

the known secretory recombinant M. ***tuberculosis*** antigen 85b, C) recombinant Tb38-1 and D) recombinant TbH-9, using protocols substantially the same as that as described in Example 3A. Total M. ***tuberculosis*** lysate, concentrated supernatant of M. ***tuberculosis*** cultures and the recombinant antigens 85b, TbH-9 and Tb38-1 were resolved on denaturing gels, immobilized on nitrocellulose membranes and duplicate. . .

DETD . . . FIGS. 3A-D, respectively, wherein the lane designations are as follows: 1) molecular weight protein standards; 2) 5 .mu.g of M. ***tuberculosis*** lysate; 3) 5 .mu.g secretory proteins; 4) 50 ng recombinant Tb38-l; 5) 50 ng recombinant TbH-9; and 6) 50 ng. . . by an arrow). These results demonstrate that Tb38-1 and TbH-9 are intracellular antigens and are not actively secreted by M. ***tuberculosis*** .

DETD . . . an intracellular antigen was confirmed by determining the reactivity of TbH-9-specific human T cell clones to recombinant TbH-9, secretory M. ***tuberculosis*** proteins and PPD. A TbH-9-specific T cell clone (designated 131TbH-9) was generated from PBMC of a healthy PPD-positive donor. The proliferative response of 131TbH-9 to secretory proteins, recombinant TbH-9 and a control M. ***tuberculosis*** antigen, TbRa11, was determined by measuring uptake of tritiated thymidine, as described in Example 1. As shown in FIG. 4A, the clone 131TbH-9 responds specifically to TbH-9, showing that TbH-9 is not a significant component of M. ***tuberculosis*** secretory proteins. FIG. 4B shows the production of IFN-.gamma. by a second TbH-9-specific T cell clone (designated PPD 800-10) prepared. . . cell clone with secretory proteins, PPD or recombinant TbH-9. These results further confirm that TbH-9 is not secreted by M. ***tuberculosis*** .

DETD An M. ***tuberculosis*** polypeptide was isolated from tuberculin purified protein derivative (PPD) as follows.

DETD . . . F. et al., Tuberculin purified protein derivative. Preparation and analyses of a large quantity for standard. The American Review of ***Tuberculosis*** 44:9-25, 1941).

DETD M. ***tuberculosis*** Rv strain was grown for 6 weeks in synthetic medium in roller bottles at 37.degree. C. Bottles containing the bacterial. . .

DETD Six fractions were collected, dried, suspended in PBS and tested individually in M. ***tuberculosis*** -infected guinea pigs for induction of delayed type hypersensitivity (DTH) reaction. One fraction was found to induce a strong DTH reaction. . . 80 .mu.l/minute. Eluent was monitored at 215 nm. Eight fractions were collected and tested for induction of DTH in M. ***tuberculosis*** -infected guinea pigs. One fraction was found to induce strong DTH of about 16 mm induration. The other fractions did not. . .

DETD Use of Representative Antigens for Diagnoses of ***Tuberculosis***

DETD This example illustrates the effectiveness of several representative polypeptides in skin tests for the diagnosis of M. ***tuberculosis*** infection.

DETD . . . 20 individuals tested, 2 were PPD negative and 18 were PPD positive. Of the PPD positive individuals, 3 had active ***tuberculosis*** , 3 had been previously infected with ***tuberculosis*** and 9 were healthy. In a second study, 13 PPD positive individuals were tested with 0.1 pg TbRa11 in either. . .

DETD Preparation and Characterization of M. ***Tuberculosis*** Fusion Proteins